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DENGUE [1]

ITS HISTORY, EPIDEMIOLOGY, MECHANISM OF TRANSMISSION, ETIOLOGY, CLINICAL MANIFESTATIONS, IMMUNITY, AND PREVENTION

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EIGHT PLATES AND TWENTY TEXT FIGURES

FOREWORD

In 1922 the Surgeon General of the United States Army recommended to the Secretary of War that a Medical Department Research Board be organized for service in the Philippine Islands for the purpose of investigating medical problems. The recommendation was carried into effect, and one of the investigations originally contemplated was that relating to the etiology and the mechanism of transmission of dengue. It was possible to begin this investigation in the early part of 1924, and the results obtained so far are recorded in this series of reports.

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[1] From the laboratories of the Bureau of Science and the Sternberg General Hospital, United States Army, Manila.

The objectives originally in mind at the beginning of the work were especially the identification, if possible, of the specific organism of dengue, and the confirmation or refutation of the claim advanced, first by Graham, and later supported by Ashburn and Craig, that the disease was transmitted by *Culex* mosquitoes. No significant advances have been made to date in the problem of specific etiology; but the second objective has, we believe, been fully attained and the question of the relationship of *Culex* to dengue settled. The observations necessary to this result led us into a more detailed study of the mosquito transmission of the disease than has hitherto been possible. The conditions under which the patient may infect the mosquito and those under which the infected mosquito may again transmit the disease to man have been investigated. We believe that these details are of value not only for dengue, but also for yellow fever, in as much as they parallel so completely the known facts in the case of yellow fever on the points that have been covered in the experiments in transmission of that disease. The unique opportunity we enjoyed of having at hand a large number of volunteers with whom to work and an ideal mosquito-proof ward for their accommodation encouraged us to go beyond the original plans and enabled us to work out, with definiteness, details only suggested or not touched upon by previous workers. Thus, in addition to a brief report concerning the work done on etiology and a complete presentation of our results in transmission by mosquitoes, studies have been made of the epidemiology of dengue and its prevalence among military personnel serving in the Philippine Islands, of the character and duration of immunity to the disease, and of the clinical characteristics shown by the group of experimental cases. We have also added an historical review of the subject of dengue and a section on prevention. These, with a comprehensive bibliography of the subject, form a whole which we hope will prove of value to future students of dengue as a fairly complete résumé of our knowledge at this time.

While some of our work has, in general, followed along lines already laid down by others, certain results may be considered of sufficient importance to be emphasized here, either because of receiving their first demonstration in our work or because of their relation to the investigation of other diseases. Among these are the following:

- The exclusion of *Culex* mosquitoes from consideration as vectors of dengue.
- The working out of the details of the mechanism of transmission by *Aëdes* Meigen.
- The demonstration of the very complete parallelism in the mechanism of transmission of dengue and of yellow fever.
- The experimental and statistical demonstration of the transient character of immunity to dengue, the outstanding difference, epidemiologically, between dengue and yellow fever.
- The demonstration of the probability of the occurrence of short-lived abortive cases of dengue, even without fever.
- The demonstration of the variability of clinical type in dengue cases produced by the same strain of virus, an observation that has its bearing on the question of the identity or diversity of the various short-lived fevers prevalent in tropical and subtropical countries.
- The application of statistical methods to the clinical study of the disease and especially of the leucocytes, the study of the latter leading to certain suggested generalizations with regard to the relationship between total leucocyte counts and differential counts that may prove valuable in the interpretation of such counts in other diseases.
- The working out of technical details in the artificial breeding of mosquitoes, especially of *Aëdes* and of *Culex*, which may prove of value to other workers along similar lines.

These points, and the others covered by our work, are presented in the following order: Historical review, transmission by mosquitoes, prevalence and distribution among military personnel serving in the Philippine Islands, etiology, clinical characteristics, prevention, immunity, bibliography, and appendices.[2]

In organizing the work a definite task was assigned to each member of the board and he became responsible for the formulation of plans in detail for the prosecution of the particular investigation entrusted to him. Prior to their initiation, proposed plans were submitted to all members of the board and other workers closely associated therewith, for criticism and elaboration.

Lieutenant Colonel Siler, president of the board, and in general charge of the work, personally directed the investigations relating to transmission by mosquitoes and prepared the section pertaining to prevalence and distribution of dengue among troops in the Philippines as well as the section on prevention; Major Hall had charge of the investigations relative to etiology and, in collaboration with Maj. P. C. Riley, Medical Corps, prepared the section relating to the clinical characteristics of dengue; Major Hitchens prepared the section constituting the historical review and the bibliography and had charge of the investigations relating to immunity.

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[2] The appendices will be included in the reprint of this report.

Dr. A. W. Sellards, Harvard Medical School, lieutenant colonel, Medical Reserve Corps, inactive, was doing reasearch work at the Bureau of Science at the time the board made these investigations and advised with us in planning the experimental work. He also studied the various microörganisms found in the viscera of normal and infected *Aëdes aegypti* Linnæus (*Stegomyia fasciata* Fabricius) in a search for a possible etiological agent.

Mr. W. Schultze, chief of the entomological section, Bureau of Science, very kindly proffered his services as consulting entomologist to the board, and Maj. P. C. Riley, Medical Corps, of the Medical Service, Sternberg General Hospital, United States Army, was consulting internist.

In addition to the above-mentioned consultants, the board was fortunate in being able to confer, in an advisory capacity, with Col. A. E. Truby, surgeon, Philippine Department, and formerly chief sanitary officer, Panama Canal Zone; Dr. Otto Schöbl, chief, division of biology and serum laboratory, Bureau of Science; and Dr. George R. Lacy, of the International Health Board.

During the course of this work, the board has occupied laboratory space in the Bureau of Science, Manila, an arrangement that has been of great value to us by reason of the close contact afforded with other workers along similar and related lines of work. We wish to express our great sense of obligation to them all and in particular to Dr. Wm. H. Brown, director of the Bureau of Science, whose active coöperation has made easy for us many things that might otherwise have been impossible of accomplishment. The same may be said with regard to the hearty coöperation of the successive commanding officers of Sternberg General Hospital, Lieut. Col. Charles F. Morse and Lieut. Col. William H. Moncrief. To them we are indebted for the opportunity to construct and maintain the mosquito-proof ward for our experimental subjects as well as for active support during the course of the work.

In reporting some of the work, more particularly that phase relating to breeding and handling mosquitoes, it has been considered desirable to recount our experiences somewhat in detail in the hope that this information will be of some service to workers who may take up similar investigative work in the future.

It will be noted that the mosquito-transmission work naturally divides itself into two very definite stages. The first is in a way purely preliminary and deals with the technic adopted for breeding and handling mosquitoes and with the administrative details connected with securing volunteers for the experimental infections. The time and the energy expended upon this phase of the work were not wasted, for it is possible that inadequate preparation is responsible for the relative failure of some of the previous dengue work. So well had every contingency been foreseen that when the second phase of the work began, that is, the transmission experiments themselves, there was never the slightest interference or delay due to lack of material or personnel.

Throughout the series of reports we have used the terms *Aëdes* (*Stegomyia*) *aegypti* and *Culex quinquefasciatus* as specific names for the mosquitoes with which we experimented. It is to be understood that *A. aegypti* is the so-called yellow-fever mosquito, most widely known as *Stegomyia fasciata*, and that *Culex quinquefasciatus* is the one that is commonly known as *C. fatigans* Wiedemann. Complete systematic descriptions of these mosquitoes are included in the Appendix.

This prefatory note would be incomplete did we fail to acknowledge our indebtedness to the military and other governmental authorities whose coöperation and interest made these investigations possible. We are especially indebted to the following officials for support and coöperation and for their willingness to place at our disposal all facilities at their command: Maj. Gen. George W. Read, commanding general, Philippine Department; Maj. Gen. James H. McRae, commanding general, Philippine Department; Col. A. E. Truby, surgeon, Philippine Department; Dr. Wm. H. Brown, director, Bureau of Science; Mr. W. Schultze, entomologist, Bureau of Science; Lieut. Col. C. F. Morse and Lieut. Col. W. H. Moncrief, commanding officers, successively, Sternberg General Hospital; Brig. Gen. Campbell King, commanding officer, Fort Mills; Col. Willis Uline, commanding officer, Thirty-first Infantry; Lieut. Col. Wm. B.

Wallace, commanding officer, First Battalion, Fifteenth Infantry; Maj. Charles J. Browne, commanding officer, Camp Nichols; Maj. J. T. H. O'Rear, commanding officer, Sixtieth Artillery Battalion, Coast Artillery Corps; and Maj. P. C. Riley, assistant to chief of Medical Service, Sternberg General Hospital.

The active coöperation and support rendered us by Lieut. Col. R. H. Pierson, Lieut. Col. F. H. Bloomhart, Capt. L. F. Wright, Capt. Wm. E. M. Devers, Capt. S. E. Brown, Capt. J. H. Whiteley, and Capt. R. Malcolm, all of the Medical Corps, in securing volunteers were greatly appreciated.

Finally, we desire to make of record our appreciation of the devotion to duty, intelligent coöperation, and attention to detail manifested by the nurses and the technical assistants associated with us in the investigations. More particularly are our thanks due to Sergt. Loverne Laycock, who was in general charge of the enlisted men of the detachment, had supervision over the property, and on the technical side acted as bacteriological assistant in the many attempts to identify the specific organism of the disease; and to Private first class Jesse F. Rhodes (laboratory specialist, second class), in general charge of breeding and handling mosquitoes, and Private first class Bonifacio Reyes (laboratory specialist, third class), in special charge of *Aëdes aegypti* breeding, both of whom discharged their exacting duties in a most efficient manner. Many thousands of laboratory-bred mosquitoes were used in the transmission experiments, and that adequate supplies were available at all times was due to the efficient performance of duty by these two men.

Our special thanks also are due to Second Lieut. Ruby Nichols and to Second Lieut. Mildred P. Carter, both of the Army Nurse Corps, for the intelligent grasp of detail and executive ability exhibited by them in the administration of the special dengue experimental ward at the Sternberg General Hospital.

HISTORY OF DENGUE

Dengue is an acute infectious disease transmitted from man to man by the mosquito *Aëdes aegypti* Linnæus. Wherever conditions for the continuous breeding of this mosquito are favorable, dengue may become endemic and, when it is introduced into a locality where conditions are temporarily favorable, or where conditions for breeding become far more favorable than usual, dengue may become epidemic. The other two factors that determine the extent of epidemics are the presence and number of cases infective to *Aëdes aegypti* and the number of exposed susceptible individuals in the community. No animal other than man has been proved to be susceptible to the infection or to act as a reservoir.

The onset of dengue is sudden and is heralded or accompanied by headache; pains in the eyes, muscles, and joints; flushing of the face; lack of appetite or actual loathing of food; and sometimes by nausea and vomiting. In the course of the disease there are typically two distinct rashes, but these are not always discoverable; in many cases the fever curve shows two definite peaks—the saddle-back curve. Relapses are not uncommon; convalescence may be protracted, and there may be a recurrence after a short interval. The period of immunity following an attack is therefore exceedingly variable in length, and an individual may have several attacks in the course of a few years. Uncomplicated dengue is rarely fatal.

NOMENCLATURE

In the literature we find many names for the disease known as dengue, and many attempts have been made to construct a derivation for the word dengue. The best discussion we have seen is the following, which is quoted from Nothnagel (pages 720 and 721):

> Opinions regarding the etymologic origin of the word "dengue" diverge considerably. According to some learned investigations, the word is of old Arabic origin, and signifies "asthenia" (Vambery), while others derive it from the East African word *"dinga,"* or from the Indian *"dangue;"* both expressions signify "blow," and perhaps are intended to designate the sudden onset of the disease. Probably, however, the word is of Spanish origin, and of similar significance to the contemporaneous expression "dandy fever." (*"Dengue,"* prudishness, affectation; *"denguero,"* affected; affected, "dandy-like.") Both expressions describe the peculiar tortuous, affected gait which the patients adopt in consequence of the pain and motor disturbances in the knees and ankles. The same symptoms are referred to in the names "polka-fever" (Brazil), "pantomime fever" (English colonies). From these pains in the knee-joints and bones dengue gets the names "broken wing," "breakbone fever" (America), "knockelkoorts," ankle fever (Dutch colonies), "abou-abous," "abou-rekabe," knee pain, "père des genoux," "des massues" (Arabia, Syria, Egypt, Tripoli). On account of the great and protracted weakness which follows the disease it was called in Philadelphia "break-heart fever;" while, on account of its mildness, it obtained the name "la piadosa," "the mild," in Spain. The expression "trancazo" points to the sudden "apoplectiform" beginning of the disease. On account of the accompanying exanthem dengue received the designations: "Fiévre rouge" (Syria), "calentura roja," "rosalia," "colorado," "giraffe," "bouquet" (viz., mottled). Its regular

occurrence at the time of the date harvest gave it the name of "date fever" in Port Said and Arabia. Very characteristic is the name of "three-day fever" formerly in use in India.

All these expressions are not the result merely of a play of words, and they point to very significant and especially to historically important characteristics of the disease.

It has also been known by the following names:

Andancio; Aboo-rikal (Graham); Abu-Rabaka; Abu-Dobus (Benghasi); Abu-ndefu; Arthrodynia.
Bucket; Baridiyabis; Bonon (Sandwich Islands).
Coup-de-barre.
Dunga.
Exanthesis arthrosia; Exanthesis rosalia; Epidemic inflammatory fever; Epidemic eruptive fever; Eruptive or exanthematous articular fever; Eruptive rheumatic fever; Exanthematous rheumatic fever.
Fievre courbaturale.
Homa Mguu.
Inflammatory fever with gastric irritation.
Knieübel; Kindinga Peop (Zanzibar).
Mal de genoux.
N'dagamonte; N'rogai (Senegambia).
Pantomime; Plantaria.
Scarlatina mitis; Scarlatina rheumatica; South Wind Fever.
Tok-kive-ana (Burma).
Zamparina.

EARLY HISTORY

Yellow fever and dengue.—Attention has been repeatedly called to the several striking points of similarity between dengue and yellow fever. Noteworthy among the contributions which have discussed the factors in which the two diseases are practically identical are those of Craig (1911, 1920). This author is of the opinion that the etiologic agent of dengue, when it is found, will prove to belong to the same group as does that of yellow fever. The work of the United States Army Medical Department Research Board has brought to light the fact that in the mechanism of their transmission also the two diseases are alike, and that the *Aëdes aegypti* mosquito is the sole transmitting agent for both.

This means, of course, that in their history these two diseases are not separable, and that they have never been endemic in regions where *Aëdes aegypti* could not propagate continuously.

The transmitting mosquito.—If the synonymy given by Dyar (1922) is accepted, we have as the earliest description of the transmitting mosquito that given by Linnæus. It was the sixth species of mosquito identified up to that time and he called it *Culex aegypti;* the locality was Egypt, and the date, 1762 (Theobald, 1910, page 617). Some entomologists still prefer the specific name *fasciatus* given to it by Fabricius in 1805; the specimen described by him came from "Americae Insulis." The mosquito *Culex calopus,* described by Meigen (see Dyar, 1922) in 1818, came from Portugal. In any case, the history of the first-mentioned specific name (Linnæus, 1762) goes back only about one and a half centuries. Subsequent descriptions of mosquitoes now considered by Dyar (1922) to be identical have been based on specimens collected in various other parts of the world. Unless the mosquitoes were breeding continuously in the localities in question they could, of course, have been only incidentally in the places where they were discovered.

The synonyms and their sources have, therefore, by themselves little value in helping us to trace the zoögeographic distribution of the transmitting agent of yellow fever and dengue. For the history of the mosquito itself there are no other repositories of information.

The earliest accurate description of dengue is not quite as old as the specific name of the transmitting mosquito—*aegypti* (Linnæus, 1762). It is interesting to note that one of the three epidemics of dengue, which occurred almost coincidently in widely separated parts of the globe, prevailed in Cairo and its environs in 1779, seventeen years after the mosquito was found in that part of the world by Linnæus. The other two accounts of dengue, which are referred to by Hirsch (1881), were those of Bylon[3] concerning an epidemic in 1779 in Batavia, Java, and of Benjamin Rush (1818), who gave an account of the epidemic in the summer of 1780 in Philadelphia.

From the standpoint of the early history of the disease (that is, of its origin or the location to which it was indigenous), these references give us no help. Yet they are the earliest accounts of dengue that we possess.

Only an entomologist would recognize the differences among mosquitoes and their habits, and *Aëdes aegypti* had traveled around the world before the eyes and the mind of Linnæus were created.

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[3] Early account of dengue, Verhandel. van het Batav. Genootsch. der Kunsten en Wetenschappen, Deel II. Cited by de Wilde, Nederl. Tijdschr. voor Geneesk. 1873. (References in Hirsch.)

Identification of dengue.—The recognition of dengue and its differentiation depended upon the fortuitous circumstance of its occurrence in localities where its symptoms were unique, and where there were also students of the type of Benjamin Rush. If dengue had been occurring previously in localities where other conditions of somewhat similar symptomatology were prevalent, and if these other conditions were far more severe and dramatic and deadly, dengue might well have remained unrecognized as a separate entity; or, if it really had been recognized, historians might have failed to pick it out had the descriptions been less clear than is the one given us by Benjamin Rush.

May it not be possible that this is the reason clear identification of dengue was forced to wait until the three factors necessary for its propagation were carried to so unusual a place as Philadelphia—a place where nothing similar had occurred before, in the memory of anyone then living?

It certainly went to Philadelphia from a locality where there were *Aëdes* mosquitoes, and the most likely place for them to be in association with such a disease was a place where there also was yellow fever. If this be true, why not examine the history of yellow fever to see if in the annals of that disease there may not be some suggestion which will give us suggestive evidence, at least, concerning the early history of dengue?

Origin of yellow fever.—In its geographical distribution, yellow fever has been confined to two relatively small endemic foci; one is about the Gulf of Mexico and the Caribbean Sea, the other the northwestern part of Africa. The earliest travel between these two points of which we know anything was the first voyage of Columbus in 1492. Though it is, of course, possible that yellow fever may have existed in two parts of the world, separated by an ocean, before human communication between the two points existed, it is more reasonable to believe that such was not the case. In discussing this question, Howard, Dyar, and Knab (1912, 1: 294, 295) have quoted Marchoux and Simond (1905), and since these authors discuss the question clearly, it seems worth while to repeat their statements here:

> It has been much discussed whether Christopher Columbus encountered yellow fever on his first or on his second voyage. But whether the 39 men left by the admiral in Santo Domingo fell victims to the disease during the summer or after the battle of Vega-Real, matters little for the history of this disease. It is certain that it existed in an endemic state

in the Antilles and along the coasts of the Gulf of Mexico before the discovery of America. It is equally certain that if the Europeans did not bring yellow fever to the New Continent they are the ones who, from Central America, have distributed it over the world. There were amongst them the most numerous victims and the new towns which were founded in the vast territory which today is the Republic of the United States had to suffer attacks. In this country alone, more than 100 epidemics were studied at different points and at different times.

Frôm New Orleans the yellow fever spread over the entire lower basin of the Mississippi and Missouri, even reaching Gallipolis [now Pittsburgh] and Cincinnati on the Ohio. One can say that it touched all the towns of the east coast from Florida to the northern frontier, and even to Quebec in Canada. On the west coast it has been seen as far up as San Francisco. New York was attacked for the first time in 1688 and had to suffer some twenty great epidemics. The yellow fever remained in an endemic condition from 1740 to 1860, and this was the same with Philadelphia, Charleston and New Orleans. All the towns of Louisiana, Mobile, Pensacola, Key West, St. Augustine, Savannah, Baltimore, Norfolk and Boston were thus greatly tried. In fact, according to what is known, it is in the United States that the yellow fever made its most frequent incursions. If one estimates at 100,000 the number of deaths of which it was the cause, one certainly remains well below the truth.

In South America it rapidly gained the Guianas and Brazil, and we see it there from 1640. The coast of this vast country, from the Amazons to Rio de Janeiro, constitutes a still persisting endemic center. From there it has made frequent incursions to Santos, and sometimes into the states to the southward, Santa Catharina, Rio Grande, reaching Montevideo and Buenos Ayres, and, going up the rivers of the La Plata to Assumption in Paraguay. On the west coast it has spread from Panama to Peru and even to Chili.

Ships, particularly the ships loaded with sugar, have transported this terrible malady to Africa where we find it in the Canaries after 1701 and perhaps even after 1501. Besides the Canaries it touched Madeira, the Cape Verde Islands, Senegal, Gambia, the Bisoagos, Sierra Leone, the Ivory Coast, the Gold Coast, Benin, Fernando Po, the mouth of the Congo and Angola with Saint Paul de Loanda. It has caused several epidemics in the island of Ascension and from this it was carried to St. Helena, but without attacking the population of the island. At this day it persists as an endemic center in the Sudanese region comprised between the Gold Coast, the Ivory Coast, the upper Senegal and the upper Niger.

Howard and his associates have also quoted Sir Rupert Boyce, who believed both the mosquitoes and the disease were indigenous to America. The quotation follows:

There is every reason for supposing that yellow fever is one of the very old diseases of mankind in the New World. It is stayed [sic] that it was known to the Aztecs under the name of *matlazahualt* [sic], and according to Humboldt it existed as early as the eleventh century.

Amongst old Spanish writers who refer to this disease may be mentioned Oviedo, who in his "Historia General de las Indias" describes the great mortality among the followers of Columbus in 1494. This mortality he attributed to the humidity of Santo Domingo, but in every probability it was yellow fever. So bad were the reports which reached Spain, that Ferdinand V. had to send out 300 convicts to the island as there were no volunteers.

Columbus in 1498, in writing to the King of Spain upon the sickness of his men, attributed their illness to "peculiarities in the air and water" in the new land. No doubt the peculiarity was the mosquito.

In the sixteenth century yellow fever is said to have decimated the Mexicans. But the first authentic history of an epidemic of yellow fever was furnished by Jean Ferreyra de Rosa at Olinda[4] in Brazil in the year 1687.

Père Dutertre, 1635, appears to have been the first to furnish details of the symptoms and progress of the disease in the West Indies. He regarded it as a new disease.

Père Labat, whose name is well known in connection with yellow fever, found on landing in Martinique in the year 1649, the disease raging in the island, the monks of the religious order stationed there being severely afflicted. The learned father stated that the disease was called "the Maladie de Siam," because in Martinique they supposed that it was imported from Siam by the ship *Oriflamme.* As, however, this ship called at Brazilian ports on the voyage, it is much more probable that either the crew became infected there or that infected mosquitoes were carried away. According to Bancroft the disease existed in St. Domingo in 1731. Old writers upon yellow fever frequently refer to the West Coast of Africa as being the original source of the disease.

Thus Dr. Chisholm believed that yellow fever was first introduced into the West Indies in 1793, when Grenada became infected from the remarkable ship *Hankey,* which had come from Bulam in West Africa. On account of this supposed origin of yellow fever it is sometimes called Bulam fever. Evidence, however, points the other way—that in fact it was a very prevalent disease in the New World, stretching from Mexico down through Central America to Brazil. Brazil appears, then, to have been the centre from which it radiated out to the West Indies. As I have stated before, the early Conquistadores suffered from it, the Latin races of the Old World being therefore the first to make its acquaintance during the time they were occupied in pushing civilization into the then newly discovered continent.

In Cuba yellow fever was probably known as the Pest or Epidemic of Havana as early as 1620. The first authentic description of the black vomit in Havana was furnished by Dr. Thomas Romay in the year 1761.

In the beginning of the eighteenth century the disease, from its appearance in various parts of Spanish America under the name of *vomito prieto,* attracted much attention, and it is particularly referred to by the historian Ulloa, who resided for some years in that country. The word *prieto* appears to be the Portuguese or nearly obsolete Spanish term for black. In Spanish the word *negro* is now universally substituted. A small pamphlet of sixty-two pages by a Dr. Gastelbondo, written at Carthagena (S. A.) in 1753 and printed at Madrid in 1755, was probably

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[4] Now a suburb of Pernambuco.—Editors. [Howard, Dyar, and Knab.]

> the first work *ex professo* on the black vomit as it appeared in South America. He gives his experience of the disease during forty years. He says on the title page that he is about to write about a disease of frequent occurrence in that part of the world, mentions change of climate and mode of living among some of the causes of the disease in newcomers, and says that the natives of Carthagena, Vera Cruz, etc., were not subject to attacks of the true black vomit fever, though liable to the "Chapetonada," a disease resembling it in some respects. [Howard, Dyar, and Knab, 1912, 1: 295, 296.]

One author, Goeldi (quoted by Howard, Dyar, and Knab, 1912), opposes the theory that America is the original home of yellow fever; he claims that it was of African origin and was imported into America at the time of the slave trade. If yellow fever had existed in Africa previous to the discovery of America, there must have been some record of it in the writings of earlier explorers of Africa, but there is nothing to support such a statement.

We may also call attention to the statements of Marchoux and Simond (1905) with regard to their observations that *Aëdes* has a certain repugnance to the black race, and the natives of Africa would therefore scarcely offer the most favorable subjects for the maintenance and spread of the disease.

Possibly the best evidence we have to support the theory that yellow fever existed in America prior to the coming of Columbus is furnished by Carlos Finlay (1912, pages 212 and 213). His account of the manner in which he came into possession of this knowledge should be told only in his own words:

> In the course of the past year, while reading a philological study on the names of "America" and "Yucatan," by the learned Bishop of Yucatan, Dr. Crescencis Carrillo y Ancona, the writer came across a remarkable phrase in a quotation from one of the *Chilam-Balam books*. These are older calendars or chronicles of the Yucatan Indians, written in the *Maya* language for the purpose of recording the principal events that affected their people. The Rev. Bishop Carrillo is known to possess a rare and valuable collection of those books, and is considered an authority on that important philological subject. His quotation from the *Chumayel manuscript* contained the following words: "There was black vomit which began to occasion deaths among us in the year 1648;" a statement which immediately suggested the idea that among the Indian manuscripts of Yucatan might be found the evidence that was wanted to prove that yellow fever was not unknown to the American Indians before the discovery. Accordingly, in the month of March of the present year, the matter was submitted to the Rev. Bishop himself, acquainting him with the state of the question and begging for information on the following points:—
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> Whether, among the Maya documents that he had examined or in the course of his other researches, any data had been met confirmatory of the

> writer's own conjectures or throwing light on the subject of the epidemics called *cocolitzle* which, according to Herrera (Decada IV., Lib. IX, Cap. VI.), used to attack the Mexican Indians on the coast of New Spain before the arrival of the Spaniards.
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> In answer to this request, the Rev. Bishop, with great courtesy and condescension, has written a most interesting and instructive letter, containing a full discussion of the subject and valuable data not to be found in our current literature.

Finlay then gives a summary of the letter in question and finally quotes the Bishop's own words.

> Notwithstanding that from this statement alone of the "Chumayel manuscript" I infer that the "vomito negro" was known to the indigenous historians, though new to the Spaniards of Yucatan, this would only constitute a conjecture, more or less grounded; whereas, what is required, as you say, is a *decisive fact.* This brings us to the essential and culminating point of the present letter.
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> *The Maya manuscripts* that I possess, like all the sacred books of the ancient *Yucatecos*, or "Chilambalam books" as they are vulgarly known, have precisely for their principal object the recording of chronological notes concerning their feasts to their gods, wars, pestilences, famines, and invasions by the Spaniards. They are chronicles and calendars. I therefore proceed to examine them, more particularly in such parts as concord with the "Chumayel" regarding the seventeenth century and the occurrence of the great epidemic, and find the following conclusive statement in the "Tzimin manuscript" (which I have so named because it proceeds from the Tzimin Indians (Tzimincah), between the folios 16 (verso) and 17:—
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> "Can ahau u buluc cit katun cu xocol tu Chichen Ytzá u hec katun ulom kuk, ulom Yaxum, ulom Ah Kantenal, ULOM XEKIK TU CAN UAC, ulom kukulcan tu pach ah Ytzaob, tu canten u than katun uale."
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> *Spanish version:* "En el 4° ahau (año maya), á la vuelta de un katun (siglo maya) que se cuenta hacia el pozo de Chichenitzá, en el asiento ó colocación de la piedra katun, llegada de kuk, llegada de Yaxum (*personajes mitológicos é históricos que daban su nombre á las épocas*), llegada de Kantenal, fué LA LLEGADA DEL VÓMITO NEGRO POR CUARTA VEZ, llegada de kukulcan después de los Ytzaes, en la cuarta colocación y significado del katun."
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> In English: "In the 4th ahau (year of the Mayas), at the expiration of a katun (*their century*), which is counted towards the well of Chichenitzá, at the placing of the stone katun, arrival of Kuk, arrival of Yaxum (*mythological and historical characters who gave their names to the epoch*), arrival of Kantenal, OCCURRED THE ARRIVAL OF BLACK VOMIT FOR THE FOURTH TIME, arrival of Kukulcan, after the Ytzaes, at the fourth placing and signification of the katun."
>
> This statement throws much light on that of the "Chumayel," for, in speaking of the same epidemic corresponding to the year 1648, it says most positively that it *was the fourth time* that it had visited this country; and considering that since the discovery in 1517 until the said year 1648 (in which the epidemic broke out, it had never been seen by the Spaniards, *it must follow that the three previous invasions had occurred before the discovery.* [These last italics ours.] [pp. 216–217.] * * *

The fact that the Indians introduced in their prognostics of evil days the threat of "vomiting of blood" is another proof that the disease had been well known to them since a long time, and precisely under its epidemic form, not as the endemic that it has now become. [p. 219.]

There seems to be no reason why we should not accept the view of Finlay and of the Bishop of Yucatan that the three epidemics previous to the year 1648 indicate that yellow fever existed there prior to the first voyage of Columbus.

The question then arises, Was yellow fever present at that time also in Africa? Upon this point Finlay quotes two writers whose opinions seem to be based on careful research. The following is quoted from pages 110 and 111 of Finlay's collected works:

El Dr. D. Antonio Pons y Codinach, en su "Tratado completo" dice: "Anteriormente al año de 1495, tres años después del descubrimiento de Cristóbal Colón, á nadie se le había ocurrido dar forma nosológica especial á ninguna de las observaciones y descripciones, y epidemias de los autores griegos, latinos, árabes ó de épocas posteriores. Desde la citada fecha, fueron por primera vez apareciendo notas, reseñas, historias y monografías, más ó menos parecidas las unas á las otras, describiendo una plaga epidémica no conocida, que se cebaba en cuantos el deber, la ambición ó el estudio obligaban á cruzar el Atlántico, en demanda de las nuevas tierras occidentales; sin que tampoco nadie formalmente pensara en encontrar asimilación la más mínima con ninguna de las afecciones antes conocidas; y lo más que se hizo al emprenderse serios estudios sobre esa enfermedad nueva, fué colocarla como una especie nueva y distinta en el género *Synochus*, peste, fiebre angioténica, tifus, etc."

Entre los autores extranjeros citaremos al Dr. Joseph Jones, catedrático de la Universidad de Louisiana, quien desde largos años viene ocupándose de la fiebre amarilla. En una Memoria leída en la Asociación médica de dicho Estado hace el referido profesor la siguiente afirmación:

"Después de un examen crítico de las obras de Herodoto, Estrabón, Justino, Cornelio Nepote, Eutropio, Plutarco, Tito Livio, Tucídides, Homero, Salustio, Virgilio, Floro, Veleyo Patérculo, César, Horacio, Ciceron, Jenofonte y Tácito, no hemos podido reconocer la enfermedad llamada hoy fiebre amarilla, en ninguna de las descripciones de epidemias particulares, ni en las alusiones á ninguna enfermedad pestilencial. Igualmente, mientras que en los escritos de la edad media tenemos descripciones de extensas y mortíferas epidemias, entre las que pueden reconocerse la peste glandular del Oriente, las viruelas, el sarampión, el tífus, la fiebre tifoidea, la enfermedad sudoral (*Sweating sickness*), la elefantiasis ó lepra, el cólera, la disentería y la meningitis cerebrospinal la *fiebre amarilla* no figura en esos anales de la historia general ni en los de medicina, antes del descubrimiento de la América por Colón." (Proceedings of the Louisiana State Medical Association, 1879, p. 54.)

Translation: Dr. Antonio Pons Codinach, in his "Tratado Completo" says: "Previous to the year 1495, three years after the discovery by Christopher Columbus, it had occurred to no one to give special nosological form to any of the observations and descriptions, and epidemics of the Greek, Roman, or Arabian authors or those of later epochs. After that date, there appeared for the first time, notes, descriptions, histories and monographs, more or less similar to one another, describing an unknown plague, which attacked by preference those whom duty, ambition or study forced to cross the Atlantic, in search of new lands in the west; without, however, anybody seriously thinking of encountering anything in the least similar to the affections already known; and the most that was done in undertaking serious studies upon this new disease, was to place it as a new and distinct species, under the generic name *Synochus*, pest, "fiebre angioténica"[5] (continued fever) typhus, etc."

Among the foreign authors we shall cite Dr. Joseph Jones, a professor in the University of Louisiana, who for many years has been interesting himself in yellow fever. In a paper read at a meeting of the medical Association of the said State, the professor makes the following statement:

> After a critical examination of the works of Herodotus, Strabo, Justinian, Cornelius Nepote, Eutropius, Plutarch, Titus Livius, Thucidides, Homer, Sallust, Virgil, Florian, Veleyo Paterculo, Caesar, Horace, Cicero, Jenofonte and Tacitus, we could not recognize the disease now called yellow fever, in none of the descriptions of definite epidemics, nor in the allusions to any pestilential disease. Equally while in the writings of the middle ages, we have descriptions of extensive deadly epidemics, among which may be recognized the glandular plague of the Orient, small pox, measles, typhus, typhoid fever, sweating sickness, elephantiasis or leprosy, cholera, dysentery, and cerebro-spinal meningitis,—*yellow fever* does not figure in those annals of general history nor in those of medicine, before the discovery of America by Columbus.

The historical information we have been able to obtain therefore all indicates that yellow fever was indigenous to those regions of America whose shores are bathed by the Gulf of Mexico and the Caribbean Sea.

Origin of dengue.—If yellow fever originated in this part of the world, then some locality in the same region must have been an early home, if not the original home, of the mosquito we now call *Aëdes aegypti,* and if this mosquito was prevalent in that neighborhood, it is rather more than likely that dengue was also prevalent. Should we not be able to find then in the references that seem to locate the place of origin of yellow fever similar information concerning dengue?

The first indication that efforts in this direction might be fruitful was the word *chapetonada,* as used by Sir Rupert

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[5] Name which Pinel proposed as a substitute for "fiebre inflamatoria" of Huxbram and Stols, "fiebre sinoca" of Hoffman and Cullen and "febris continua non putrida" of Boerhaave, which that author attributed to irritation and tension of the vessels.

Boyce (quoted by Howard, Dyar, and Knab, 1912). This word is not contained in any of the English dictionaries, but the French and Spanish generally define it as an "acclimatization fever." The Spanish Enciclopedia Universal Ilustrada (1908) gives more detailed information, and locates the condition in Peru. We quote the following:

> *Chapetonada*—La primera enfermedad que padecen los europeos después de haber llegado al Perú, ocasionada de la mudanza del clima.
>
> *Translation.*—The first illness that attacks Europeans after arriving in Peru, caused by the change of climate.

It is entirely evident from Boyce's use of the word and from the definition given in other Spanish dictionaries that *chapetonada* referred to the acclimatization fever suffered by Europeans in any of the warmer countries of South America. The term could scarcely have meant always a mild form of yellow fever, although it is entirely likely that it did at times. It would scarcely have been used for malaria, because the symptoms of that disease were well known. None of the intestinal diseases would be likely to have been called acclimatization fevers; they would have been given names more descriptive of them. Persons coming to Manila from regions where dengue is not endemic usually must pass through an acclimatization fever, and that *chapetonada* is dengue.

The difficulties in the way of picking out the mild disease dengue from the midst of mild attacks of the severe disease yellow fever are well illustrated by the attitude of Boyce in his discussion of yellow fever in Africa. In the early days, as when Boyce visited Africa, mild yellow fever was all too often called inflammatory fever or bilious remittent fever, and the true nature of the beginning epidemic forced it to be recognized only by the occurrence of true black vomit or death. There is a parallel that troubles us equally to-day: sometimes we make the diagnosis "chicken pox" but treat the illness administratively as "smallpox." Of the actual existence of the mild disease there can be no doubt and, although we cannot agree with him altogether, the exact words of Boyce are most interesting in our present discussion. He says, on pages 358 and 359:

> * * * The "inflammatory fever" of the West Indies has been long recognized as an acclimatizing seasoning fever, which newcomers were expected to get. It was another name of some local or endemic malady about which no one knew anything except that all were expected to get it, at one time or another.

Bilious Remittent Fever.—This fever takes the place in West Africa of the inflammatory fever of the West Indies. It is a very old name, and typified the "acclimatizing," "seasoning," or "endemic" fever of any locality which newcomers were expected to get. Volumes have been written upon it. Some of the older writers held that, just as in inflammatory fever, it could pass into severe yellow fever; others held that it was a specific entity; others, again, that bilious remittent fever carried from one locality could burst out into the malignant yellow fever on arrival in another country. All these doubts and theories pointed to the fact that the older clinicians were observing one and the same disease, but in different types of severity. From investigations of careful records of outbreaks of yellow fever there can be no other conclusion than that a very large number of cases of genuine yellow fever have been classed as "bilious remittent fever."

Basing his conclusions upon the writings of the older Spanish authors on the Americas, Carlos Finlay gives us definite information concerning the epidemic diseases which afflicted the early settlers. On pages 132 and 133 of his collected works is the following:

El año de 1648 una terrible pestilencia atacó las Antillas francesas de San Cristóbal y de Guadalupe, colonizadas por esa nación: en 1627 la primera, y en 1635 la segunda. Otras enfermedades habían agoviado á los nuevos colonos desde los primeros años de su venida á esas islas, y en particular, una que con el nombre de "*Coup de barre*" describe Du Tertre y que ha sido generalmente considerada como la fiebre amarilla. Pero, á la verdad, los términos en que el escrupuloso misionero francés menciona ese mal no parecen referirse á una enfermedad tan mortífera ni tan desastrosa como la fiebre amarilla epidémica; y el mismo Du Tertre tiene el cuidado de advertir que la *peste* que invadió esas islas en el año de 1648 era, hasta entonces, desconocida allí. ¿Podrá ser que ya en aquellos tiempos, como ha resultado después en las islas de Guadalupe y Martinica, existiese una fiebre amarilla benigna (*frusta*), la fiebre inflamatoria de B. Féraud, que alternara con explosiones más ó menos espaciadas de fiebre amarilla maligna ó epidémica?

Translation.—In the year 1648 a pestilence attacked the French Antilles of San Cristóbal and Guadalupe, colonized by this nation: in 1627 the first, and in 1635 the second. Other diseases afflicted the new colonists from the first years of their coming to these islands, and in particular, one of them which was described by Du Tertre with the name "*Coup de barre*" and which has generally been considered yellow fever. However, as a matter of fact, the terms in which this scrupulous French missionary mentions this disease do not appear to refer to a disease so fatal or so disastrous as epidemic yellow fever; and Du Tertre takes care to add that the *plague* which invaded these islands in the year 1648 was, until then, unknown there.—May it be that at that time, as has resulted since in the islands of Guadalupe and Martinique, there existed a benign (*frusta*) yellow fever, the inflammatory fever of B. Féraud, which alternated with explosions of malignant or epidemic yellow fever at longer or shorter intervals?

On pages 138 and 139 this information is further extended as follows:

> La "fiebre amarilla epidémica," la remitente maligna ó pútrida de origen palúdeo, la fiebre biliosa inflamatoria de las Antillas, y el Matzahuatl—son las únicas enfermedades epidémicas, endémicas ó pseudo-epidémicas, que podrían, quizás, llenar algunas de las condiciones que he señalado en las primeras pestilencias americanas. Digo que no conozco otras, porque no tengo que ocuparme de las fiebres eruptivas, que fueron importados probablemente de Europa y, en todo caso, eran bien conocidas por los primeros exploradores españoles, ni tampoco de la *peste bubónica* ni de la meningitis cerebro espinal epidémica, que jamás han sido observadas hasta ahora en la América tropical.
>
> El Matzahuatl ataca principalmente á la raza cobriza, no á los europeos, y tiene su asiento de predilección en las elevadas mesetas de la América Central y meridional (v. g. la capital de México); por lo tanto, queda excluída de nuestra consideración.
>
> La "fiebre biliosa inflamatoria de las Antillas" á cuya enfermedad Bérenger Féraud fué el primero que asignó un lugar apropiado en los cuadros nosológicos de estas regiones amarilígenas, viene á ser una "fiebre amarilla frusta," que, á pesar del sindrome, á veces alarmante, que la acompaña, no pasa de ser una enfermedad de índole esencialmente benigna; quedando ya por esta sola circunstancia, excluida de entre las que pudieron ocasionar las mortíferas pestilencias que destruían la tercera parte ó la mitad de los atacados, y causaban la muerte del tercero al quinto día.
>
> La fiebre remitente palúdea, maligna ó pútrida, existe con el carácter de endemia, á veces formidable, tanto en las Antillas y otros focos amarilígenos, como en el resto de las Américas, en Europa, en Africa y en el Asia, particularmente en la India inglesa, en el delta del Ganges, donde jamás se ha conocido la fiebre amarilla. Esta última circunstancia ha permitido diferenciarla, no tan sólo de la misma fiebre amarilla sí que también de la fiebre inflamatoria de las Antillas, con la cual suele aún confundirse, designándose ambas con la misma diversidad de apelativos. Distínguese, empero, por su distinto comportamiento bajo la influencia de la quinina, por la poca tendencia de la afección palúdea á ser transportada por las vías marítimas y por no transmitirse de los enfermos á los sanos. En la India inglesa, lejos de toda influencia amarilígena, suelen observarse, sin embargo, casos de remitente maligna ó pútrida, principalmente en los europeos cuando llegan atacados del escorbuto antes de recibir la intoxicación palúdica, que ofrecen un cuadro sintomático muy parecido al del "vómito negro" de las Antillas. * * *
>
> *Translation.*—"Epidemic yellow fever," the malignant or putrid remittent of malarial origin, inflammatory bilious fever of the Antilles, and matzahuatl—are the only epidemic, endemic, or pseudo-epidemic diseases which could, perhaps, fulfill some of the conditions which I mentioned, of the first American pestilences. I say that I do not know others,

because I do not have to occupy myself with the eruptive fevers, which were probably brought from Europe and, in any case, were well known by the first Spanish explorers, nor with the bubonic plague nor with epidemic cerebro-spinal meningitis, which have never been observed up to the present in tropical America.

The matzahuatl attacks principally the copper-colored race, not the Europeans, and has its seat of predilection in the elevated plateaus of central and southern America (e. g., the capital of Mexico);—for which reason it is excluded from our consideration.

The "inflammatory bilious fever of the Antilles," to which disease Bérenger Féraud was the first to assign an appropriate place in the nosologic tables of these yellow-fever regions, seems to be an "abortive yellow fever," which, in spite of the syndrome, at times alarming, that accompanies it, is no more than a disease of essentially benign nature; being therefore for this sole reason excluded from among those diseases which could occasion the deadly pestilences that destroyed a third or a half of those attacked, and caused death on the third to the fifth day.

Remittent malignant or putrid malarial fever possesses the character of an endemic, at times formidable, as well in the Antilles and other yellow fever foci as in the rest of the Americas, in Europe, in Africa, and in Asia,—particularly in British India, in the delta of the Ganges, where yellow fever has never been known. This last circumstance has permitted its differentiation, not only from yellow fever itself, but also from the inflammatory fever of the Antilles with which it is still confounded, both being designated by the same diversity of names. It is distinguished, however, by its characteristic behavior under the influence of quinine, by the slight tendency of the malarial affection to be transported by maritime routes, and by its not being transmitted from the sick to the well. In British India, far from all yellow-fever influence, there are observed, however, cases of malignant or putrid remittent, principally in Europeans when they arrive with scurvy before receiving the malarial intoxication, which offers a symptomatic picture very similar to the black vomit of the Antilles.

Among the conclusions to this paper is the following, page 141:

6.° En fin, queda en pie la sospecha de que la enfermedad designada con el nombre de "*coup de barre*" por el P. Du Tertre, que afligió á los primeros colonos franceses desde su primera ocupación de las Antillas menores, no fuese la "fiebre amarilla epidémica" tal como allí mismo estalló en 1648, sino la "*fiebre inflamatoria*" ó "*fiebre amarilla frusta*" que aun suele reinar en esas islas durante los intervalos que median entre los períodos epidémicos de *fiebre amarilla verdadera.*

Translation.—Finally, there remains the suspicion that the disease designated by the name "coup de barre" by P. Du Tertre, and which afflicted the first French colonists from their earliest occupation of the Lesser Antilles, was not the epidemic yellow fever such as broke out there in 1648, but the "inflammatory fever" or "abortive yellow fever" which still reigns in those islands during the intervals between the epidemic periods of true yellow fever.

In "*coup de barre*" or in "*fiebre amarilla frusta*" we undoubtedly have dengue. Abortive yellow fever or inflammatory bilious fever, or yellow fever without the striking symptoms of jaundice and black vomit, might at times be mild yellow fever, and there might be a great many cases of it during an epidemic of yellow fever; but, if an epidemic of this condition occurred between epidemics of yellow fever, it is likely that it was something else, and that something else could scarcely have been anything but dengue.

We believe then that the historical evidence we have found gives us good ground for constructing the theory that dengue as well as yellow fever originated in tropical America.

Evolution of the virus.—It is quite natural at this stage of our knowledge, considering the great similarity between dengue and yellow fever, to wonder whether or not they had in the beginning a common origin. If the original virus was like that of yellow fever, did one strain of it become weakened by passage through persons who possessed some degree of immunity or by some other process? Or was the original virus weak, like that of dengue, and did one strain of it become greatly increased in virulence, possibly through attacking successively a large series of persons devoid of immunity? Or was the change possibly a sudden one?

Early records of epidemics.—As noted above, the earliest accurate accounts of dengue concern epidemics in widely separated parts of the world that occurred in 1779 and 1780. If we wish to take a critical attitude with regard to the identity of the diseases then described, we may cite the recent work of Van der Velde (1923) and of Verwoort (1923) with regard to a febrile disease which they have described as occurring in Sumatra. Their disease, which they believe to be different from any hitherto described, nevertheless presents some points of similarity to dengue. It is a fever of short duration, apparently accompanied by little or no mortality. The type and degree of the fever in their cases present little that is distinctive; its usual duration is from four to seven days; it is usually of a continuous or a remittent type, the saddle-back curve appearing rather infrequently. As in dengue, injection of the eyes is an early symptom, but the exanthem occurs in only a small proportion of the cases. Leucopenia is not characteristic of their cases. Further points in which their cases differ from the usual conception of dengue are the large proportion of cases showing albuminuria (60 to 80 per cent) and the frequent occurrence of bronchitis and of splenic enlargement. Enlargement of the lymph nodes is very exceptional. They have isolated from the blood of a representative number of these cases an organism which they described as indistinguishable morphologically from those found in yellow fever and in infectious jaundice, and to which they have given the name *Leptospira pyrogenes.*

They believe that their disease, "spirochaetosis febrilis," is distinct from dengue. Whether it is indeed a clinical entity or whether further study will show it to be a composite clinical group made up of cases of infectious jaundice, dengue, and possibly other fevers, only time will show. They report icterus in a small proportion of their cases and have isolated their *Leptospira* from the urine of some patients. The clinical characteristics given, and especially the fact that their cases seem to have been drawn exclusively from the native population, render it improbable that the group as a whole should be classed as dengue. The recognition of such a disease as they describe, in the same part of the world as the epidemic originally described by Bylon, permits the suggestion that Bylon's cases may have been of the same character. Bylon said the symptoms were almost the same as those of early cases of plague, the patients complaining of headache, lassitude, and pains in the joints, but that they were rid of it in three days, and there were no bad consequences.

The disease described by Gaberti as existing in Cairo in 1779 possessed certain clinical features which are not common in present-day dengue epidemics. He mentions chiefly the knee involvement and gives as a name for this condition "knee-disease." He also states that there was swelling of the fingers and that the pains continued for more than a month. Stitt (1913) is of the opinion that the sudden onset and the swelling would seem to belong to relapsing fever as much as to dengue. Sandwith (Medical Diseases of Egypt) states that bone pain, chiefly of the knee, is the symptom of which Egyptian natives with relapsing fever most frequently complain.

The description given by Benjamin Rush (1818) of the Philadelphia epidemic of 1780 is not susceptible of such differences of interpretation as are these accounts. If we analyze carefully the statements of Rush, it is easy to see, as Stitt points out, that there are included in the description cases of typhoid fever ("hemorrhage from the bowels about the eleventh day"); malaria ("the exacerbations were more severe every other day"); and probably dysentery ("the fever was sometimes accompanied by symptoms of dysentery"). Notwithstanding these complicating factors, the description of Rush gives us a clear picture of the striking and characteristic features of dengue. The following is quoted from Rush's original paper (page 239):

> July and August were uncommonly warm. The mercury stood on the 6th of August at 94½, on the 15th of the same month at 95°, and for several days afterwards at 90°. * * *
>
> The moschetoes were uncommonly numerous during the autumn. A certain sign (says Dr. Lind) of an unwholesome atmosphere.
>
> The remitting fever made its first appearance in July and August, but the symptoms were so mild, and its extent so confined, that it excited no apprehensions of its subsequent more general prevalence throughout the city.
>
> On the 19th of August the air became suddenly very cool. Many hundred people in the city complained, the next day, of different degrees of indisposition, from a sense of lassitude to a fever of the remitting type. This was the signal of the epidemic. The weather continued cool during the remaining part of the month, and during the whole month of September. From the exposure of the district of Southwark (which is often distinguished by the name of the *Hill*) to the south-west winde, the fever made its first appearance in that appendage of the city. Scarcely a family, and in many families, scarcely a member of them, escaped it. From the Hill it gradually traveled along the second street from the Delaware, improperly called Front-street. For a while it was confined to this street only, after it entered the city, and hence it was called by some people the *Front-Street fever*. It gradually spread through other parts of the city, but with very different degrees of violence. * * *
>
> All ages and both sexes were affected by this fever. Seven of the practitioners of physic were confined by it nearly at the same time. The city during the prevalence of the fever was filled with an unusual number of strangers, many of whom, particularly the Friends (whose yearly meeting was held in the month of September), were affected by it. No other febrile disease was observed during this time in the city.
>
> This fever generally came on with rigor, but seldom with a regular chilly fit, and often without any sensation of cold. In some persons it was introduced by a slight sore throat, and in others by a hoarseness which was mistaken for a common cold. A giddiness in the head was the forerunner of the disease in some people. This giddiness attacked so suddenly, as to produce, in several instances, a faintness, and even symptoms of apoplexy. It was remarkable that all those persons who were affected in this violent manner recovered in two or three days.

I met with one instance of this fever attacking with coma, and another with convulsions, and with many instances, in which it was introduced by a delirium.

The pains which accompanied this fever were exquisitely severe in the head, back, and limbs. The pains in the head were sometimes in the back parts of it, and at other times they occupied only the eyeballs. In some people the pains were so acute in their backs and hips, that they could not lie in bed. In others, the pains affected the neck and arms, so as to produce in one instance a difficulty of moving the fingers of the right hand. They all complained more or less of a soreness in the seats of these pains, particularly when they occupied the head and eyeballs. A few complained of their flesh being sore to the touch, in every part of the body. From these circumstances, the disease was sometimes believed to be a rheumatism; but its more general name among all classes of people was, the *break-bone fever.*

I met with one case of pain in the back, and another of an acute ear-ache, both of which returned periodically every night, and without any fever.

A nausea universally, and in some instances, a vomiting, accompanied by a disagreeable taste in the mouth, attended this fever. The bowels were, in most cases regular, except where the disease fell with its whole force upon them, producing a dysentery. * * *

A rash often appeared on the third and fourth days, which proved favourable. This rash was accompanied, in some cases, by a burning in the palms of the hands, and soles of the feet. Many people at this time, who were not confined to their beds, and some who had no fever, had an efflorescence on their skins. * * *

About the beginning of October the weather became cool, accompanied by rain and an easterly wind. This cool and wet weather continued for four days. The mercury in the thermometer fell to 60°, and fires became agreeable. From this time the fever evidently declined, or was accompanied by inflammatory symptoms. * * * [pp. 231–235.]

The convalescence from this disease was marked by a number of extraordinary symptoms, which rendered patients the subjects of medical attention for many days after the pulse became perfectly regular, and after the crisis of the disease.

A bitter taste in the mouth, accompanied by a yellow colour on the tongue, continued for near a week.

Most of those who recovered complained of nausea, and a total want of appetite. A faintness, especially upon sitting up in bed, or in a chair, followed this fever. A weakness in the knees was universal. I met with two patients, who were most sensible of this weakness in the right knee. An inflammation in one eye, and in some instances in both eyes, occurred in several patients after their recovery.

But the most remarkable symptom of the convalescence from this fever, was an uncommon dejection of the spirits. I attended two young ladies, who shed tears while they vented their complaints of their sickness and weakness. One of them very aptly proposed to me to change the name of the disease, and to call it, in its present stages, instead of the break-bone the *break-heart fever.*

In discussing the treatment, Rush makes one statement that seems to have remained unnoticed by those who have written upon the history of dengue. He says, on page 236:

> I discharged the bile entirely by means of the lenient purges that have been mentioned. In this practice I had the example of Dr. Cleghorn, who prescribed purges with great success in a fever of the same kind in Minorca, with that which has been described.

In a footnote the name of this fever is given as "The tertiana interposita remissione tantum of Dr. Cullen." It would be interesting to learn how closely this remittent tertian resembles that described by Rush. Did dengue actually exist on the Island of Minorca previous to 1780?

Distribution of dengue.—Subsequent to the three outbreaks we have been discussing, commonly considered the earliest recorded in the history of dengue, and which, we wish to repeat, occurred almost simultaneously in widely separated parts of the globe, epidemics of dengue began to attract general attention. There are now well-known endemic centers throughout the Tropics where the conditions for the continuous breeding of *Aëdes aegypti* are favorable.

Compared with yellow fever, dengue has become very widespread, and we have been much interested in attempting to explain this rather curious difference. It is not difficult to understand how *Aëdes* mosquitoes can be carried from one port to another, and conditions for their transportation were more favorable in the early days than they are to-day.

Water butts were not always well protected, and occasional small collections of water on wooden sailing vessels were the rule rather than the exception. In Public Health Reports (1904), there is a report of an "Epidemic of dengue in the Territory of Hawaii during 1903," which was reported by Assistant Surgeon Wilson, at Honolulu, in part as follows:

> During the late summer of 1902 dengue was reported in Shanghai and other Chinese ports frequented by steamers calling at Honolulu, Hawaii. Two or three of the regular passenger liners, on arriving at Honolulu from the Orient at this time, reported having had a few cases of the disease soon after leaving Chinese ports. One of these vessels in particular, the steamship *Doric*, arrived September 11, having had on the voyage 12 cases of dengue, as reported by the ship's surgeon. One of these cases had the attack a few days before reaching Honolulu, but on his arrival had neither fever nor rash. This vessel was twenty-three days from Hongkong, a few days less from Shanghai, and ten days from Yokohama.

> The first cases of dengue recognized as such in the Hawaiian Islands occurred in Honolulu about the 1st of January, 1903. For several weeks previously, however, cases of doubtful measles and scarlet fever had been treated by physicians. From all that can be ascertained of the situation, and the writer was in Honolulu during all the time, it is very likely that several cases of dengue occurred sufficient to act as a link between cases, convalescent or mild, arriving from the Orient during the autumn of 1902 and undoubted dengue cases first reported early in January, 1903. This opinion is further borne out by the fact that quite a number, if not a majority, of the physicians of Honolulu had never seen dengue and in addition were not on the lookout for it, principally because dengue recognized as such had never been known in the Hawaiian Islands. However this may be, suffice it to say that dengue in the islands was first reported from Honolulu early in January, 1903, and two or three months thereafter had been reported by government physicians from all the other islands of the group. During April and May the epidemic appeared to have reached its maximum in Honolulu and has been gradually waning since until at present, December 1, 1903, only an occasional case is reported. [pp. 67–68.]

After a voyage requiring several weeks a vessel would be rather likely to reach port free from yellow fever, because this disease either destroys its victims without delay or the patients recover and are immune. Furthermore, patients with yellow fever are less likely to move about than are those with dengue, since from 10 to 20 per cent, or possibly more, of the persons who have dengue do not permit it to interrupt their regular routine, and thereby may infect an enormous number of mosquitoes. This, of course, is not the case with yellow fever, and such difference alone accounts for the more rapid spread of dengue and the greater number of cases within a given time in an epidemic.

Another peculiarity of dengue favorable to its maintenance during long voyages is its tendency to relapse and the fact that persons may have second and third attacks within a relatively short period.

Epidemics.—Many writers have catalogued and discussed the important epidemics of dengue, but nowhere have we found a more interesting account than in Nothnagel (1905, pages 721 to 723), from which the following is quoted:

> 1779–1800:
>
> The first reports concerning dengue originate from the year 1779, during which it was observed at Java (Batavia), and about the same time at Cairo and Alexandria. During the years following, the reports of epidemic outbreaks accumulated. The disease prevailed in 1780 upon the coasts of Coromandel, of Arabia, and Persia.

Even at that time the disease made an isolated excursion over the subtropical zone, and during the hot summer of 1780 broke out suddenly in Philadelphia (40° N. Lat.).

During the year 1784 it appeared for the first time in Europe with epidemic outbreaks in Cadiz (36.5° N. Lat.) and Seville (37.5° N. Lat.). Most probably the disease was carried thither by troopships which came from the West Indies. A second epidemic broke out in Cadiz in 1788. At the end of the previous century dengue was reported from Grenada (Lesser Antilles).

1818:

Epidemic in Lima (Peru).

1824–1828:

With the years 1824–1825 a geographically extensive period of dengue begins, which gradually spread over a great portion of the tropical and subtropical zone.

In the years 1826–1828 dengue spread itself in extensive epidemics over the greater portion of West India, the Virginian Isles, and the Greater and Lesser Antilles (Havana, 1826; St. Thomas, 1827).

From here numerous coast towns of Central America and of the most southern portion of North America and the most northern portion of South America were attacked (Havana, Charleston, New Orleans, Vera Cruz, the coast of Florida, and Venezuela). Isolated cases are alleged to have been observed at that time in New York and Boston, and notably again in Philadelphia, among the crew of a ship coming from Cuba.

1830–1870:

During these four decades dengue is met with in large and small epidemics at numerous places in its tropical and subtropical area of distribution. The principal sites of the disease were:

Numerous coast towns of India (1830, 1835–1836, 1844–1847, 1853–1854).

Tahiti and other South Sea Islands (1852–1853).

The coast of Arabia and northern Egypt (Alexandria, Cairo, Port Said). The principal epidemic years here were 1835–1836, 1845, 1868.

Furthermore:

Tripoli (1856), Cyprus, and Syria (1861).

Réunion (1851), Zanzibar and Madagascar (1864), Senegambia and Greece (1845–1848, 1856, 1865), the Canary Islands (1856).

Numerous West Indian Islands (1860–1863).

Central America, especially New Orleans, Savannah, Charleston, Iberville, New Iberia, Georgia and Louisiana, Mobile, the coast of Texas and Ohio (1839, 1844, 1848, 1850).

South America, Peru (1852), and Rio de Janeiro (1846–1848).

In Europe: Cadiz (1863, 1867).

Two facts stand out during this period in the geographic distribution of dengue. To begin with, its first important appearance in South America, namely, in Rio de Janeiro, where from 1846–1848 the disease prevailed yearly "on water and on land" during the hot season (December and January), and each time with considerable severity. Secondly is to be noticed that, during this period, Europe was twice attacked, and, as on the previous occasions of 1784, so too in 1863 and 1867, the harbor city of Cadiz in southern Spain became the center of an epidemic, which in both instances was brought in by troops returning from West India, the home of dengue. From Cadiz the disease spread to Xeres, Seville, and a few other cities of Andalusia, but the north remained exempt; in fact, in Spain, as a whole, the disease remained insignificant.

1871–1873:

A very extensive outbreak of dengue occurred in the years 1871–1873, when it appeared first on the East African coast (Zanzibar), then on the Arabian coast (Aden, Jiddah, Mecca, Medina, and Tanyef), and in Port Said. From here it was carried by an emigrant steamer to Java, from Aden by a troop-ship direct to Bombay, Kananur, and Calcutta. Following on this it spread through the countries of the Indian Ocean, Hindustan, especially in Calcutta and Madras, Rangoon, China, Formosa, Java, Celebes, and Sumatra. The last appearances in this important epidemic period occurred on the Persian coast, in the islands of Mauritius and Réunion, situated on the coast of eastern Africa, and, furthermore, in Tripoli and Senegambia. In the same year, 1873, epidemics took place in the southern portion of North America bordering on the Gulf of Mexico, Louisiana, Alabama, etc.

1876–1888:

During the year 1876 there was an epidemic in Hongkong. During the year 1878 the disease was brought to Ismailia (Suez Canal) by a pilgrim ship, and from this place it traveled to Alexandria and up the Nile. In the same year Malta was also infected by a troop-ship from India. We further add: 1880, the ports of the Red Sea, especially Jiddah; 1880–1881, Cairo; 1881, Khania, to Crete and Syria; 1885–1886, the Fiji Islands; 1887, Gibraltar; 1888, Cyprus, and in the same year "whole Virginia" (Charlottesville).

Within this period fall numerous epidemic outbreaks in Syria, especially in Beirut, where according to de Drum and Suquet, no fewer than fourteen epidemic years could be counted from 1861 to 1889.

1889:

Of particular interest is the considerable distribution of dengue immediately preceding the influenza pandemic of 1889; in Syria and Palestine, in Asia Minor, Cyprus, Rhodes, Chios, and the islands of the Archipelago; furthermore, in Cairo and Ismailia. The disease spread "with incredible rapidity," and attacked now for the first time Damascus and Jerusalem.

From Beirut, Smyrna and Maghnissa were infected, and from the latter place Constantinople, and soon afterward the port of Piraeus and Athens. From Constantinople the disease was carried to the Pontine southern coast (Trapezunt) and to Varna and further still to Salonica.

On this occasion dengue continued even into the cool season (November), and in some places was almost continuous with the succeeding

> influenza. To this fact much importance must be attached; for if influenza and dengue were identical diseases, as they have so often erroneously been maintained to be, they would not have succeeded each other in such a short time—in Constantinople, Athens, and Salonica. But since dengue is a specific infectious disease *sui generis*, it also did not render the inhabitants of these cities immune against the approaching influenza coming from the north. Also in the Fiji Islands in 1885 there existed an influenza and a dengue epidemic closely following and even overlapping each other.
>
> 1890–1895:
>
> During these periods only a few endemic outbreaks are known. They affected Senegambia (St. Louis) in 1890, in 1893 the East Indian fleet, and in 1895 Hongkong.

Texas was again visited by dengue in 1897.

Subsequent epidemics that have been noted in the Public Health Reports of the United States Public Health Service are as follows:

1899: Adalia, Turkey.
1901: Hongkong.
1902: Canton, China.
1903: Honolulu; Fort Jefferson, Florida.
1904: Cotulla, Texas; Miami, Florida; Shanghai.
1905: Brisbane, Australia.
1906: Pascagoula, Mississippi; Havana and Matanzas, Cuba.
1907: Brownsville and Laredo, Texas; Cartagena, Columbia.
1908: Miami, Florida; Salina Cruz, Mexico; Amoy, China.
1911: Amoy, China.
1912: Florida; Amoy, China.
1913: Progreso, Mexico; Amoy, China; Savannah, Georgia.
1914: Iquique, Chile.
1915: Beirut and vicinity of Tripoli; Turkey in Asia; Bermuda; San Juan, Porto Rico; Laredo, Texas.
1916: Argentina; Hawaii; Santurce, Porto Rico; Austin, Brownsville, and Eagle Pass, Texas.
1918: Dominican Republic; Louisiana; Chefoo, China; St. Thomas, Virgin Islands; Galveston, Texas; Shanghai, China.
1919: Key West and Florida at large; Louisiana; Woodville, Mississippi; Vera Cruz, Mexico; Charleston, South Carolina.
1920: Coffee County and Alabama at large; Key West, Florida; Savannah, Georgia; Galveston, Texas.

Period of clinical study.—The literature on the epoch that can be called that of clinical and epidemiological study is rich in information concerning symptomatology and the factors discussed in the present report, in the section on immunity; that is, relapses, recurrences, the relative immunity of persons coming from endemic areas as compared with natives of places visited by dengue for the first time, and other points that may be elicited by clinical observation. A few of these, illustrative of the group, have been referred to in the section on immunity.

PERIOD OF INVESTIGATION

Transmission.—To Graham (1903) belongs the credit for having first brought forward positive evidence of mosquito transmission of dengue. The observations leading up to his experiments were made locally and apparently quite independently. Even in the earlier literature, however, we find numerous suggestions from which it is now easy to derive the opinion that dengue could scarcely be transmitted in any other way.

It will be remembered that Benjamin Rush noted the unusual presence of mosquitoes in the hot summer of 1780 in Philadelphia. Hirsch (1881) quotes Waring[6] as saying of the epidemic of 1826, "The break-bone fever has been suppressed by the frost;" and of that of 1828, "It terminates under the effect of frost." He likewise quotes Arnold[7] as follows:

> This disease is undoubtedly affected by frost. The diminution of cases last fall was as marked as the diminution of the cases in our endemic climate (yellow fever) usually is.

Subsequent to Graham, reports upon observations indicating mosquito transmission became more specific. An epidemic on the French ship *Kersaint* was reported by Cazamain (1916), who suggested the possibility of mosquitoes acting as carriers of the disease. Such evidence as that furnished by Ross (1906) is most striking. Dengue, as well as malaria, was prevalent in Port Said, but when Ross (1908) had eradicated the mosquitoes, in his successful antimalaria campaign there, dengue likewise disappeared. McCulloch (1918) had a similar experience on Corregidor Island. After the island had been freed of mosquitoes dengue, even when it was introduced, did not spread. Welch (1923) found that dengue in 1922 did not visit towns in Alabama that had been rid of mosquitoes. Hanabusa (1917), on the other hand, reported an outbreak of dengue in a regiment of engineers in Formosa at a time when mosquitoes were not prevalent; eighty-six men were attacked in a group of eight hunderd forty-two. With regard to the dengue on Formosa Island, Koizumi, Yamaguchi, and Tonomura (1917) incriminated the mosquitoes *Stegomyia scutellaris* and *Desvoidea obturbans;* they found no indication that it had been transmitted by *Culex fatigans*. These authors admit that their volunteers were not under observation and confinement before the experiment began, and this part of their work needs confirmation before the mosquitoes noted can be considered seriously as having played a rôle in the transmission of dengue. Furthermore, we may remark here that the results of their injections of virus blood into guinea pigs, combined with certain other peculiarities, have led us to wonder if the epidemic with which they were working was in fact identical with the dengue that has been studied by us.

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[6] N. Am. Med. & Surg. Journ. (1830).

[7] Charleston Med. Journ. (1857).

Graham on mosquito transmission.—We believe the experiments in transmission made by Graham in 1903 should be described in his own words and that the paragraph that precedes his description should be included. It will be noted that he says the city was terribly infested with *Culex fatigans* (now, *Culex quinquefasciatus*) and *Stegomyia fasciata* (now, *Aëdes aegypti*). For some reason he feels so certain *Culex quinquefasciatus* was the transmitting mosquito that throughout his work he practically disregards the presence of *Aëdes aegypti* (*Stegomyia fasciata*). There can be no doubt, now, that in each of his successful experiments there were *Aëdes* among the *Culex* mosquitoes he used for producing the infection. Graham says on pages 210, 211, and 214:

> I saw something of the epidemic which prevailed on the Syrian Coast in 1889, and my experience gained at that time, together with what I had learned from the reports of other observers in different epidemics, inclined me to the belief that in the mosquito was to be found the factor which made the dengue in certain localities very contagious, while not at all in others. Between Beyrouth and the mountains to the south exists an immense sand-bank; to the north irrigated mulberry orchards. The houses in the city are without exception badly infested with mosquitoes. With but few exceptions the villages dotting the slopes of Lebanon contain them in greater or less numbers. In the city of Beyrouth itself none of the forms of *Anopheles* have been found, although careful search has been made for them by others as well as myself. The city, however, is terribly infested with *Culex fatigans* and *Stegomyia fasciata*. I have searched carefully a considerable number of the villages on the slopes of Lebanon facing the city, and have found the *Culex fatigans* in almost all of them, even to a height of 5,000 feet above the sea-level. I have also found *Stegomyia fasciata*, although less frequently.
>
> At the beginning of the epidemic I commenced a series of experiments in order to determine, if possible, whether the *Culex fatigans*, which exists in perfect swarms in the city, could carry the dengue from person to person.

(1) The first experiment was done with a mother and her nursing child. The day the mother was taken with the initial chill all the mosquitoes in one room were killed by generating chlorine gas, and each day after she entered another room in which the mosquitoes had been so killed. During the fifteen days' time she was under observation three rooms were used, the room after being freed from mosquitoes being each time protected as well as possible from their re-entrance. The mother, who was extremely anxious to protect her son of 8 months from the disease, submitted to the annoyance and trouble. The child was allowed to nurse from his mother during the whole attack of the fever, which was a severe and typical one, and escaped any observable form of infection.

(2) Experiment No. 2 was carried out in the same way, and exactly the same precautions were taken to keep out the mosquitoes. There were four small children who slept in the same bed on the floor, their ages ranging from 4 to 11 years. The eldest of the four contracted the fever and the other three played in the same room where he lay sick and slept in the same bed with him at night, no one of the three contracting the disease. The rooms were kept free from mosquitoes during thirteen days.

(3) In the third experiment the father of three children contracted the disease in the city, and was willing to submit to almost any inconvenience to protect his wife and children. The same precautions were taken as in Nos. 1 and 2. The man had a severe attack lasting five days, accompanied by high fever, eruption, and desquamation. The rooms were kept as free as possible from mosquitoes during seventeen days, with the result that no other member of the family was taken ill.

In several other cases I tried the same experiment, but gave it up because of carelessness on the part of the household, which resulted in my finding mosquitoes in the rooms when I made my visit of inspection. They said the dengue was better than the confinement. However, in those cases where care was taken the results were all that could be expected.

The evidence, however, in these experiments was of a negative kind, and in order to obtain something more positive, several experiments were done in direct inoculation or infection. My excuse for exposing *men* to infection was that everyone in the city not immune to the disease was going to get it anyway, also that the disease was entirely without danger to life in young and healthy men. The character of the experiment was explained in each case, and the young man, for only young men were used, expressed his entire willingness to have the "aboo-rikal," as it is called, for the sake of the money offered. The difficulty was not to find one willing to undergo the experiment, but to select the best ones from the many who offered. Four men were selected, inmates of houses in which no case of dengue had as yet occurred. In each case several mosquitoes were taken from the netting of patients sick with the fever and put inside the netting of the candidate for the disease, he sleeping night after night with the mosquito in the netting. In one case the initial chill came on five days after the mosquitoes were put in, six days afterwards in a second, and four days after in the third. In the fourth case, no attack having occurred at the end of a week, another

> batch of infected mosquitoes were put in, with a negative result. This young man gave the history of having had a severe attack of dengue accompanied by eruption and desquamation during the epidemic of 1889. Possibly he was immune for that reason. During that epidemic I had a very severe attack, and during this last one I escaped although constantly exposed, and although I took no means to protect myself. In all of these cases the men were under agreement to remain in their houses in order not to contract the disease in any other way. In a city where so infectious a disease was raging I still felt that these three men might have received the contagion in some other way. In order to avoid this objection mosquitoes were taken from inside the netting of a dengue patient and carried up to a village on the mountain slope where as yet no case had occurred. After visiting my patient for the infected mosquitoes I changed my clothing and took a bath before mounting my horse to ascend the mountain. In this village, about 3,000 feet above the sea, there are *almost* no mosquitoes. It is dry and very healthy. I easily found two young men in different parts of the village who consented for a consideration to take their chances of having the dengue. One of them, after sleeping four nights under the netting with the mosquitoes, was taken with a severe typical attack of dengue. The other had his initial chill after having passed five nights in the company of the mosquitoes. These men continued to sleep under the mosquito nettings I had prepared for them until some time after they were well, all the mosquitoes in the netting having been killed to avoid infection of other people. No other cases of dengue occurred in this village during the summer that I could learn of, although I made the most careful inquiry.
>
> * * * Mrs. Graham undertook one day to procure a mosquito for me from one of the cages, and while holding the killing bottle over the mosquito to be caught, did not notice until too late that she was being bitten by another. The third day after she was taken to bed with severe nausea and vomiting and high fever, the attack running the course of a typical sharp attack of dengue. This was her first attack of dengue, and there had occurred at no time during the summer any cases in the house, neither had she been anywhere else to expose herself to this disease. The mosquitoes in this case had drawn blood from the dengue patient fifteen days previous to the time when she was bitten. * * *

These quotations show with what cleverness Graham planned his experiments. It is unfortunate that the types of mosquitoes to which his volunteers were exposed were not better controlled. Furthermore, the species of the mosquito that bit Mrs. Graham is not stated. It is not surprising, considering Graham's successful results and the statements he made concerning them, that some of those who subsequently took up the study of dengue transmission by mosquitoes should have thought first of attempting transmission by *Culex quinquefasciatus*.

Carpenter and Sutton (1905), however, made experiments in transmission with several different mosquitoes, but the results were uniformly negative. They used *Culex stimulans* Walker, *Culex tarsalis* Coq., and *Aëdes aegypti,* but did not try *Culex quinquefasciatus.* Their mosquitoes were bred in the laboratory and kept for from five to fourteen days after biting. Finally all were dissected and carefully examined. Carpenter and Sutton considered their mosquito transmission experiments untrustworthy and thought that no definite statements could be made concerning the results. The details of only four of their attempts at mosquito transmission are given; two of these were negative, one was positive after six days, and one after two weeks from the date of biting. The volunteer subjects were unfortunately not protected at all times from the bites of other mosquitoes.

The mosquito transmission experiments of Bancroft (1906) were carried out in a dengue-infected district; this, as pointed out by Cleland and Bradley, may operate in two ways. His apparently successful cases might have acquired the disease in some other way, or the failures may have been due to previously acquired immunity among his volunteers. Two of his volunteers came down with dengue. Both were bitten by *Aëdes aegypti*—one twelve and one ten days after the mosquitoes had bitten dengue patients. In the experiments that resulted negatively the volunteers were bitten fifteen, fifteen, and seventeen days after the mosquitoes had bitten the dengue patients. Bancroft made the interesting observation that persons, who lived in noninfected districts in the country, and visited in the daytime friends in town who were ill with dengue, acquired the disease. He deduces from this that if dengue really is a mosquito-borne disease, the transmitting agent must be a day-biting species. This naturally rules out *Culex quinquefasciatus* and incriminates *Aëdes aegypti.*

Guiteras and Cartaya (1906) mentioned some experiments in mosquito transmission, using *Culex pipiens,* but they published none of the details, stating merely that they attached very little importance to their work, although convinced that dengue, like yellow fever, is a mosquito-borne disease.

Agramonte (1906), working in Havana, made unsuccessful attempts to transmit dengue by mosquitoes. He used several species and had them bite volunteers at various intervals after they had been fed upon dengue patients. He suggested that his failure was due to some undiscoverable fault in technic. He notes that *Culex quinquefasciatus* was present in Havana at that time. His clinical observations led R. A. O'Brien (1908) to incriminate *Stegomyia notoscripta* as a carrier of dengue, since the distribution of this mosquito was coincident with the epidemic of which he writes.

Ashburn and Craig.—Ashburn and Craig (1907) studied an epidemic of dengue that occurred at Fort William McKinley, about 14 kilometers (9 miles) from Manila. For their volunteers, they were forced to use men who had already passed, apparently unaffected, through an epidemic at that place. The result was that of nine who were exposed to the bites of mosquitoes three were immune, one may have had a slight attack of dengue before, and three possessed relative immunity "for while they developed dengue from the inoculation of a comparatively large amount of dengue blood, the symptoms were mild in character, and in one case the incubation period was greatly prolonged." One of their volunteers was found upon subsequent injection of virus to be susceptible to dengue, but he had seemed to be immune; the mosquitoes refused to bite him under any of the conditions to which he was exposed. The details of the experiments of Ashburn and Craig, we believe, should be quoted (pages 129 to 131):

> *The mosquito used.*—In looking over the geographical distribution of dengue and various species of mosquitoes, we found but one species (*Culex fatigans* Wied.,) of this insect that apparently occurred wherever dengue did. * * * In Theobald's monograph the map illustrating the known distribution of *Culex fatigans* Wied. might almost be used to illustrate the distribution of dengue fever, and if to this map be added the regions in which this mosquito has been demonstrated since it was published, the association of dengue and *Culex fatigans* Wied., is still more striking.
>
> For this reason, and because this mosquito was employed by Graham in his experiments, we decided to work with this species at first, and in the event of our results being negative, to extend our work to embrace other species.
>
> We have used mosquitoes reared in captivity, and also those caught in natural surroundings. However, in our successful case produced by the mosquito, we used mosquitoes reared by us from the egg, and thus we are sure that no infection occurred in these insects before they bit the dengue patients.
>
> Our mosquito experiments were conducted as follows: The patient suffering from dengue was placed in a bed beneath a mosquito net in a mosquito-proof tent. At night from twenty to thirty mosquitoes were liberated beneath the mosquito bar and collected in the morning; almost invariably all the mosquitoes left alive had bitten and were full of blood. The subject to be experimented upon, having been placed in bed beneath a mosquito net in another mosquito-proof tent, the mosquitoes which had bitten the dengue case the night before were liberated beneath his

mosquito net, and orders given that the man remain beneath the net until the mosquitoes had disappeared; later we allowed the man to remain out of bed during the day, the mosquitoes being kept beneath the spread net. With one exception, which has been noted, all the men were bitten a few times, but in most instances the mosquitoes died before the men had been bitten severely. We also confined mosquitoes that had bitten dengue cases in glass jars, and kept them as long as from four to six days before allowing them to bite, but in the few instances in which we tried this method, our results were all negative.

Experiment No. 11

Case 11, * * *—B. L. W., private, Hospital Corps, United States Army. This man had been on duty at the Division Hospital for several weeks, and as no cases of dengue had occurred in the hospital, had not been exposed to the disease, so far as we could determine. On September 12, 1906, the man being in good health, he was placed under a mosquito net with mosquitoes that had bitten case 88 * * * on the night of September 11. Case 88 was suffering at the time from a typical attack of dengue. Case 11 was not bitten by mosquitoes until the night of September 13, and developed no symptoms until the night of the 17th, but upon reference to his chart it will be seen that he had fever for nearly twenty-four hours before he noticed any symptoms. If we assume the period of incubation to be the period intervening between the 13th, the night upon which he was first bitten, and the 16th, when he had his first rise in temperature, the incubation period would be about three days and one-half. However, if we assume the disease to have commenced when he first noticed symptoms—that is, upon the evening of the 17th—the incubation period would be a little over four days. The following is a summary of his clinical history:

September 12: Put under net with mosquitoes that bit Case 88 last night.

September 13: Bitten by mosquitoes last night.

September 18: Had headache and felt uncomfortable last evening. This morning complains of headache and a dull pain in the articulations.

September 19: Still complains of headache and general muscular pain and soreness. His face and eyes are greatly congested.

September 20: Last night had severe pain in the head, eyes, and the muscles of the back, but feels much better this morning.

September 21: Is feeling better. A faint rash is visible covering the chest and abdomen.

September 22: Complains of soreness and stiffness in the muscles. The eruption is now plainly visible and typical of dengue.

September 24: Feels well. The eruption has almost disappeared.

October 1: Returned to duty.

Remarks.—This case was in every way typical of a moderately severe attack of dengue. The symptoms were those seen in the great majority of naturally acquired infections and the temperature chart is a very characteristic one. This man had not been exposed in our dengue camp

> before being bitten by the mosquitoes, and did not leave the mosquito-proof camp until after the onset of the disease.
>
> For reasons which have been stated, of the nine men exposed to the bites of infected mosquitoes, only four can be considered in estimating the results obtained. Of these, one, or 25 per cent, developed a typical attack of dengue following the bites of infected mosquitoes; but we do not consider that the three negative cases are of much value, as the conditions were such as to cause some doubt as to whether the men were bitten.

These authors have never felt entirely satisfied with the results of their work in mosquito transmission, and Colonel Craig has attempted on numerous occasions to make it possible to do or to have done such work as this board has been able to accomplish.

Cleland, Bradley, and McDonald.—Cleland, Bradley, and McDonald (1916, 1918, and 1919) were fortunate in being able to conduct their experimental transmission work in a place where dengue did not exist. In March, 1916, an extensive epidemic of dengue was prevalent in Queensland, and reached some of the north coast towns of New South Wales. These authors realized that if infective material could be successfully transported to Sydney where the disease had never been known to spread, any results obtained would be free from fallacies attendant upon experiments made in an endemic or epidemic area. They considered that, since both *Culex quinquefasciatus* and *Aëdes aegypti* were common household mosquitoes in most parts of Australia, it was probable that, if dengue is transmitted by a mosquito, it must be one or the other or both of these. In view of the earlier experiments of Bancroft (1906) in Australia, greater suspicion fell on *Aëdes aegypti.* However, they used both mosquitoes in their experimental work.

Among other mosquitoes which they considered were *Scutomyia notoscripta* Skuse, which is widely distributed through Australia, although they never met it in any abundance; it resembles *Aëdes aegypti* very closely, but can be distinguished from it by a white band on the proboscis; *Culicelsa vigulas* Skuse, a common bush mosquito; *Culicelsa annulirostris* Skuse, which is widely distributed in Australia and is present in the affected area, but there seems to be no reason to consider it responsible for the conveyance of any disease to man; and *Nyssorhynchus annulipes* Walker, a transmitter of malaria in Australia, which appears to be widely distributed there.

The first series of mosquito-transmission experiments made by these writers resulted negatively; four volunteers were bitten by *Aëdes aegypti,* and two by *Culex quinquefasciatus.* The details of the second series of mosquito experiments can be given most accurately by quoting from their report (pages 342 to 345):

> In our second series of experiments mosquitoes were collected in Mullumbimby and the surrounding district, about 100 *S. fasciata* and 112 *C. fatigans* being thus obtained. The insects were collected from the hotel at which we stayed in Mullumbimby, from the post office, and from private houses in the town and district in which dengue fever cases had occurred—in some cases from the actual bedroom where patients were lying sick with the disease. A few mosquitoes were caught on the journey from Brisbane to Mullumbimby. * * *
>
> The *Stegomyia fasciata* and *Culex fatigans* were transferred to special cages, one containing *S. fasciata,* the other *C. fatigans.* The cages were made with a rounded opening, to which was attached a net sleeve. Through this the hand could be passed to add freshly caught mosquitoes and for the biting experiments.
>
> At Mullumbimby, on May 8th, 1916, the dengue patient (X) who became ill on May 7th, was bitten by the *Stegomyia* then in the cage; on the 9th, he was again bitten by *Stegomyia,* and on the evening of the 8th, he was bitten by *Culex.* Both species of mosquitoes bit this patient well, and thus many of the Mullumbimby district mosquitoes had certainly been fed on the blood of a dengue case in the acute stage. Exactly how many mosquitoes bit this patient it is impossible to say, as this part of the work was conducted in a badly-lit bedroom.
>
> On May 11th, 1916, these mosquitoes arrived in Sydney.
>
> Biting experiments with the *Stegomyia* were conducted on May 11th, 12th, 13th, and 14th, and with *Culex* on May 11th, 12th, 13th, and 14th, as shown more clearly later.
>
> On May 15th, some seven *Culex* and eleven *Stegomyia* collected in the Grafton district, chiefly from houses of dengue patients, were added. Over 112 mosquitoes were collected in Grafton, of the following species: *Stegomyia fasciata,* twenty-seven; *Culex fatigans,* forty-six; *Culicelsa annulirostris,* thirty-seven; *Nyssorhynchus annulipes,* two. But although all care was taken, the mortality between Grafton and Sydney was large, and hence only this small number was added to the boxes.
>
> Further feeding experiments were made with the remaining mixed Mullumbimby and Grafton mosquitoes, viz. with *S. fasciata* on May 15th, 16th, 17th, 18th, 19th, and 23rd; with *C. fatigans* on May 15th and 16th.
>
> On June 29th, the cages used in the experiments were emptied of the dead mosquitoes, and the remaining bodies that were not crushed were examined separately with a hand lens. Seventy-five *Stegomyia* were counted (two being males) in the *Stegomyia* cage; no other mosquitoes were found in this cage. Seventy-eight *C. fatigans* (two being males) and one *Culex annulirostris* were found in the *Culex* cage.
>
> This procedure forms an additional check by another observer (J. B. C.) that the classification of mosquitoes was made accurately by B. B., and although about twenty-five *Stegomyia* were unaccounted for—probably they had been unrecognisably crushed in travelling, etc.—we can be

reasonably certain that no *Culex* was included in the *Stegomyia* cage with which we obtained our positive results.

The following is a short summary of the experiments made and results obtained with mosquitoes, taking the nine persons volunteering *seriatum:*

Case I. J. G., male, laboratory assistant (18 years), the subject of an unsuccessful *Stegomyia* biting experiment of the first series, was bitten on May 11th, 1916, at 2.15 p. m., by some twenty-eight *Stegomyia.* He remained well until the afternoon of the 19th, eight days later, when he noticed he had headache. That evening at 7 p. m. (eight days and five hours) he was again bitten by *Stegomyia,* and, while sitting with his hand in the cage, first became definitely ill. He passed through a typical attack of dengue fever, showing a double temperature curve, rash, and symptoms described in detail below. Blood from this case reproduced the disease on injection. ***Result positive.***

Case II. McD., male, laboratory assistant, not previously the subject of experiment, was bitten on May 12th, by ten *Stegomyia,* and on the 18th by three or four *Stegomyia.* He remained well until June 3rd, seventeen days from the second biting and twenty-two days from the first biting, when he had an influenzal attack with coryza for a few days, with no rash and nothing suggestive of dengue. ***Result negative.***

Case III. G., male, laboratory assistant, not previously the subject of experiment, bitten by about nine *Stegomyia* on May 13th, 1916, and by about three *Stegomyia* on the 17th. No symptoms have followed these bitings to date—July 14, 1916. ***Results negative.***

Case IV. Wm., male, laboratory assistant, not previously the subject of experiment, was bitten by about thirty-six *Stegomyia* on May 14th, 1916, (mid-day), and by about thirty-six *Stegomyia* on the 15th (12.30 p. m. and 4.30 p. m.). On the 20th (six days and nine hours from first biting), whilst going to bed at night, he became ill and had a typical attack of dengue, with double temperature, rash and other symptoms. * * * His blood on injection reproduced the disease. ***Result positive.***

Case V. M., female, a nurse, was bitten by eighteen *Stegomyia* on May 16th, 1916 (noon) and became ill on the 25th, 10 p. m. (nine days ten hours later), and passed through a rather severe type of dengue with marked rash and double temperature. No blood was taken from this case for injection experiments. ***Result positive.***

Case VI. B. B., medical practitioner, was in dengue fever districts—Mullumbimby, Casino and Grafton, leaving Grafton for Sydney by boat on May 13th, 1916. To keep the mixed Grafton mosquitoes alive, he allowed them to bite him on the 12th and 14th, but remained perfectly well till the 23rd (2 p. m.); ten days after leaving the dengue district, he was bitten by fifteen *Stegomyia.* He remained well till May 29th, and his temperature was normal till May 31st, on rising at 9 a. m. (seven days and nineteen hours), he then became definitely ill and passed through a severe attack of dengue, with definite prodromal and secondary rashes, double temperature, and marked pains, etc., * * * Blood from this case reproduced the disease on injection. *Result positive,* but open to criticism since B. B. had been in a dengue district eighteen days before the attack developed.

Case VII. W. T., bitten by one *Stegomyia* on May 12th, 1916. No illness followed. *Result negative.*

Case VIII. M., a patient at a hospital, was bitten by *Culex fatigans* as follows: on May 11th, 1916, by about twelve; on May 12th and 13th by an unknown number; on May 14th, by at least twenty. *Result negative.*

Case IX. J. O. S., laboratory assistant, a subject of *Culex* experiment in first series, was bitten by *C. fatigans* as follows: On May 15th, 1916, by two; on May 19th, by an unrecorded number. No symptoms followed. *Result negative.*

In discussing the above results, it is important for the reader to bear in mind that our main object was to determine whether either or both of the mosquitoes experimented with were capable of transmitting infection. We were quite in the dark, even if one or both species of mosquito were a transmitter of the disease, about a number of other important circumstances connected with such a possible means of transmission. The mosquito, if it carried infection at all, might or might not need a period to elapse after biting a patient before it became able to infect another person, and might remain infective for a period quite undetermined by us. Hence mosquitoes collected might not prove successful transmitters, not because they could not carry infection, but because they were not for one reason or another "ripe." Therefore, although our mosquitoes were collected in a district where dengue was prevalent, some from houses where patients were actually ill, and many from houses where patients had recently been ill, we felt it advisable to increase the chance of getting results by letting them bite patient *X.*, who had acquired the disease in the usual manner, on the dates mentioned. We were not in a position, and did not try, to solve the question of the "ripening" period, if any, nor of the period during which the mosquitoes remained infective.

Again, we deemed it advisable to have our first volunteers bitten more than once, and that because of the uncertainty as to whether the mosquitoes had "ripened," especially if infected from the known bitten patient, and because of the unknown time which the mosquitoes might remain infective. We foresaw that, to a certain extent, these multiple bitings might complicate our results and prevent us from obtaining the exact incubation period, but we attempted to arrange the experiments in such a way that we might hope to elucidate this point. As it turned out, the double biting has actually only interfered with the understanding of the incubation period in one case (*Wm.*), and the later volunteers, being only bitten once, tend to confirm, in this case, the longer incubation period rather than the five days five hours period which may have been the incubation period for *Wm.*

The deductions that can clearly be drawn from these experiments are that *Aëdes aegypti* (*Stegomyia fasciata*) is capable of transmitting dengue, whereas no evidence is obtained from the two cases bitten by it that *Culex quinquefasciatus* (*C. fatigans*) could transmit the disease. No attempt was made in these experiments to ascertain whether or not the virus in the mosquito had to pass through any ripening period. The mosquitoes used were caught in houses where there were cases of dengue and nobody could tell the time of their initial infection.

Chandler and Rice.—During the summer and fall of 1922, there occurred in parts of Texas a scourge of yellow-fever mosquitoes (*Aëdes aegypti*) such as had not before been recorded and, accompanying it, an epidemic of dengue which attacked between 500,000 and 600,000 people.

Chandler and Rice (1923) took advantage of this opportunity to make etiological investigations. Their observations concerning the prevalence of mosquitoes showed that in no part of the state where there was dengue was *Aëdes aegypti* absent, and their study of the relation between *Aëdes aegypti* and the epidemiology of the outbreak led them to the conclusion that, in that epidemic, this mosquito was the primary factor in transmission. They did not feel prepared to say that *Culex quinquefasciatus* could not, under some circumstances, transmit the disease; but they believed the evidence they had adduced was sufficient to show that this mosquito plays, at most, a minor part. In order to prove conclusively that *Aëdes aegypti* was really the transmitter of dengue in this epidemic, they planned a number of transmission experiments. Various circumstances prevented their following completely the program laid out, but they were able to make valuable observations. The following is their own account of their transmission experiments (pages 249, 250, 257, and 258):

> For the Galveston experiments volunteers were found among new arrivals on evening trains, and were immediately placed in carefully screened rooms, where they were observed for ten days, during which time blood counts, urine analyses, and Wassermann tests were made. Except for minor noninfectious injuries, all the volunteers were found to be in good physical condition. Two women had come direct from Colorado Springs, Colo., two men from Denver, Colo., one man from Albuquerque, New Mexico, two from Yellowstone Park, and one from Leadville, Colo., the statements of the volunteers being corroborated by baggage checks or Pullman slips * * *. The experiments were performed at a private house where good and intelligent coöperation was received. The copper screens were doubled with mosquito bar, and every one watched for mosquitoes indoors. A total of three were seen and killed during the course of the experiments. None of the volunteers left the second floor, but they visited each other on that floor, and had their meals served in their rooms. The laboratory-bred mosquitoes used in the experiments were released under a closely gathered net with the volunteer stripped to the waist, 30 to 40 mosquitoes being used in each instance. The mosquitoes were seen to bite in every case recorded as a bite, the sting was noted by the volunteer, and a papule was observed; we cannot, of course, be certain that in some instances the same mosquito did not bite twice. After a number of bites had been recorded, all the mosquitoes were killed.

> *Summary of experiments.*
>
> Dengue fever was successfully transmitted to 4 out of 6 volunteers by *Aëdes aegypti,* as follows:
>
> *Case 1.* Twenty-four hours after biting patient in second day of disease. Incubation period approximately five days and eighteen hours.
>
> *Case 2.* Forty-eight hours after biting patient in third day of disease. Incubation period six days and four hours.
>
> *Case 3.* Seventy-two hours after biting patient in fourth day of disease. Incubation period six days and twelve hours.
>
> *Case 4.* Ninety-six hours after biting patient in fifth day of disease. Incubation period four days and two hours.
>
> Of the 2 volunteers to whom the disease was not transmitted by infected mosquitoes, one (no. 5) was bitten by mosquitoes fed ninety-six hours previously on a patient on the fifth day of the disease and again nine days later by mosquitoes fed twenty-four hours previously on a patient in the second day of the disease. Finally, five days later, he was intravenously inoculated with 1 c. c. of blood from no. 4 taken about forty-four hours after onset of the disease, but was not infected and did not subsequently contract the disease. The other unsuccessful case (no. 6) was bitten by mosquitoes fed ninety-six hours previously on a patient in the fifth day of the disease. He had a mild attack of the disease twenty-seven days after being released. * * *
>
> While these experiments were carried on in an epidemic area, and are therefore not so absolutely conclusive as they would have been if they could have been carried on in an uninfected place, we feel reasonably certain that the results can be relied upon in view of the precautions which were taken to prevent outside infection. We conclude, therefore, that the disease, as it appeared in the Texas epidemic, was transmissible by *Aëdes aegypti* in from twenty-four to at least ninety-six hours after biting a patient, and that these mosquitoes can become infected from patients in various stages of the disease from the first to at least the fifth day * * *.

It will be noted that, in the transmission work done by this board, mosquitoes successfully transmitted dengue only when the period between the biting of the patient and the biting of the volunteer was more than ten days. We obtained no evidence that the mosquito is ever merely a mechanical agent. The results of our work lead us to believe that in dengue, as in yellow fever, the virus must remain in the mosquito for about eleven days before the insect is capable of transmitting the disease. In view of our results, we find it difficult to understand and explain the successful results obtained in mosquito transmission by Chandler and Rice.

CONTAGIOUSNESS OF DENGUE

In order to obtain definite evidence of the contagiousness of dengue, Ashburn and Craig (1907) made such experiments as

Reed and his associates had made in Cuba to demonstrate the exclusive transmission of yellow fever by the mosquito.

At the hospital at Fort William McKinley, efforts were made to produce dengue by exposure of healthy men to fomites. The men used in this experiment lived in mosquito-proof tents with patients suffering with dengue, through the entire course of the disease. They slept in the same beds with the patients, wore the underclothing and pajamas of the patients, and ate and drank from the same table utensils. Eight men were exposed in this way, and since none developed the disease from such contact, the conclusion was drawn that dengue is not a contagious disease, and that dengue patients may, therefore, be placed in the general wards of a hospital without fear of infecting others, provided precautions are taken to protect them from mosquitoes.

Other methods were used by Cleland, Bradley, and McDonald (1919) to transmit the virus from one person to another. They failed to infect a volunteer through the application of infective serum to a scarified area on the skin. The result was negative when infective material was applied upon swabs to the mucous membranes of the nostrils. A very doubtful, and probably negative result, followed the gargling of the throat with infective material, followed by swallowing the same.

INCUBATION PERIOD

We have, in the literature, clinical reports of attacks of dengue occurring at definite times following probable infection. Such observations as those based upon outbreaks of dengue among groups of sailors after arrival of a ship at an infected port are probably accurate, but they cannot have the definiteness possessed by the recorded time intervals in experimentally produced infections under controlled conditions.

The first case appeared among those on board the steamship *Kersaint* at the infected port of Saigon, after the fourth day subsequent to arrival at that port. Hare (1898) tells of two men who visited an infected town on a certain day and remained there only during that day; both came down with dengue five days later. Of the twenty men who came down with dengue after returning from the infected island of Rouad, Adrien (1918) gives the time of attack as follows: Three on the fourth day; seven on the fifth; five on the sixth; three on the eighth; and two on the tenth.

The incubation periods in the cases produced experimentally and reported in the literature up to 1923 are summarized by Armstrong (1923, page 6) as follows:

Experimenter.	Number of cases.	Incubation period, days.	Experimenter.	Number of cases.	Incubation period, days.
Graham	2	4	Cleland et al	2	7–8
Do	2	5	Do	1	9–10
Do	1	6	Ashburn and Craig	1	3–4
Bancroft	1	5	Chandler and Rice	1	4–5
Do	1	6	Do	1	5–6
Cleland et al	1	6–7	Do	2	6–7

In the cases produced by the injection of blood, the following results were obtained:

Experimenter.	Number of cases.	Incubation period, days.	Experimenter.	Number of cases.	Incubation period, days.
Cleland et al	1	4–5	Cleland et al	1	15
Do	4	5–6	Ashburn and Craig	4	2–3
Do	3	6–7	Do	2	3–4
Do	3	7–8	Do	1	4–5
Do	5	8–9	Do	1	7
Do	2	9–10	Chandler and Rice	2	5–6

The longest incubation period of which there is any record is fifteen days, reported by Cleland, Bradley, and McDonald (1919). This case is commented upon in the clinical section of our report.

The incubation periods of the cases produced in the work of this board are discussed in the section of this report devoted to the clinical manifestations of dengue.

The virus.—In support of his evidence concerning the transmission of dengue, Graham (1903) attempted to demonstrate the presence of the virus in his potentially infected mosquitoes. He injected a suspension of the salivary glands of mosquitoes, which he believed to be infected, under the skin of a volunteer. The mosquitoes had bitten a patient with dengue twenty-seven days before. On the third day after the injection, the patient had a chill and high fever with an attack "resembling dengue in every way." The attack was so severe, however, Graham did no further work along that line. He thought it was not septicæmia, because he found his dengue parasites in the blood.

The finding of dengue parasites in the blood of the patient is not now taken seriously, and the diagnosis in this case remains an open question. The suspicion seems persistent that possibly the material injected was not free from the common pyogenic bacteria.

Ashburn and Craig (1907) were the first to demonstrate experimentally the presence of infective virus in the circulating blood. They injected eleven men with the blood of patients suffering with dengue, seven of whom subsequently developed dengue. The result in the rest was doubtful. Three of the four later proved to be immune.

These authors were likewise the first to show that the virus of dengue is filtrable. The technic used and the results of their filtration experiments should be given in their own words (pages 123, 124, and 128).

> Having proven by our inoculation experiments with unfiltered blood from dengue patients that the disease could be thus transmitted and, furthermore, that while the cause must, therefore, be present in the blood, it is not possible to demonstrate it in either fresh or stained specimens by any known method of examination, we are forced to the conclusion that the causative organism must be ultramicroscopic, as in the case of yellow fever, rinderpest, etc.
>
> FILTERS USED AND CONTROL METHODS
>
> In our filtration experiments we have employed a Lilliput diatomaceous filter, which was tested each time before it was used. Before using, the filter was sterilized and the filtration done under 730 millimeters pressure.
>
> After filtering the blood the following control test of the filter was made in each case. A suspension, in nutrient bouillon, was made of *M. melitensis* and *S. cholerae*, and then filtered through the filter used in filtering the blood; the filtrate was then incubated for two weeks, daily examinations of it being made. The filter in use retained both these organisms, the filtrate remaining sterile for two weeks when it was thrown away. In the control filtrations the same filter was used, after careful sterilization, as was employed for the dengue blood, and the same pressure was maintained during filtration.
>
> Besides the control test of the filter, we kept in each case a portion of the filtered dengue blood for a period of ten days, making daily examinations, and in one case, several cultures in bouillon. No growth was obtained in either the filtered blood or the cultures.
>
> We regard these two cases of dengue produced by the intravenous injection of filtered dengue blood as the most typical cases of the severe type of the disease which we have observed and we believe that these two experiments prove conclusively that dengue can be transmitted by blood which has been passed through a filter which retains organisms as small as 0.4 μ in diameter (the measurement of *M. melitensis*). It also proves that in all probability the causative agent is ultramicroscopic in size, for the reason that neither in fresh nor stained blood smears nor in the filtrate obtained from dengue blood, can any organism be demonstrated with the microscope. It may be possible that in some other fluid or organ of the body, or in some phase of its life history in an insect, the organism may be visible, for Novy, in his work upon *T. lewisi*, has

> proved that even so large a parasite as this trypanosoma may exist in a form so small in cultures that it passes through a Berkefeld filter. While this may prove to be true as regards the dengue organism, we feel justified in stating, that, so far as present evidence goes, the organism causing dengue is ultramicroscopic in size. This conclusion explains the uniformly negative results obtained by nearly every trained observer in the search for a dengue parasite.
>
> We conclude that an organism is present in the filtrate, rather than a toxin, because of the length of the period intervening between inoculation and the appearance of clinical symptoms, and also because we have reproduced the disease by inoculation of the blood of experimental cases.

The distribution of the virus in the blood was the subject of careful observations by Cleland, Bradley, and McDonald (1919). Through the inoculation of susceptible persons they first confirmed the findings of Ashburn and Craig (1907) that the virus is present in the blood. Then they set up experiments to ascertain in what element of the blood the virus resides. They showed that it is present in the serum of clotted infective blood. Three volunteers received subcutaneous injections of washed corpuscles from cases of dengue. The corpuscles were obtained by withdrawing blood from a vein and forcing it immediately into a solution of sodium citrate in normal saline solution. The mixture was centrifuged and the corpuscles rewashed four to seven times. Two of the volunteers injected with the suspension of washed corpuscles gave negative results and one a result not quite conclusive. One of the volunteers giving a negative result received corpuscles from blood taken on the third day of the disease and the corpuscles were injected within twelve hours. Serum from the same sample caused a definite attack of dengue, showing that the blood itself was infective. The other negative case received corpuscles from blood taken on the fourth day of the disease and injected the day following its collection. The citrated plasma in this case also gave a negative result. The volunteer, who was possibly positive, was injected with corpuscles from blood taken on the second day of the disease and injected the following day. The illness was a mild one, beginning about five days twenty hours after the injection. The patient's appearance and symptoms were those of a person with a mild attack of dengue.

They also found that the virus may be present in the blood as early as eighteen hours and as late as ninety hours after the onset of dengue. It was not found in the blood one hundred thirty and one hundred ninety hours after the onset. It should be pointed out that the last results were obtained through the inoculation of blood. Compared with the amount of virus taken up by a mosquito the quantities used were enormous. Infection of a volunteer might result from the injection of 0.5 cubic centimeter of blood taken at a period of the disease when relatively little virus remained in the circulating blood—too little to infect a mosquito, except by the merest chance. The work of this board indicates that mosquitoes may become infected from a patient during the period beginning a few hours before the onset of symptoms and through the second day of the disease. A certain proportion may become infected on the third day, but by the fourth day, or subsequently, infection would be the exception. We lack information concerning the presence of virus in persons whose period of convalescence is greatly protracted. Deductive evidence indicates that the continuation or endemicity of dengue does not depend on carriers of the virus.

Cleland, Bradley, and McDonald (1919) further made observations upon the permanence of the virus outside the body. In preserving virus blood intended for injection they usually kept it in the refrigerator, but frequently it was transferred from the laboratory to the hospital without special precautions. In one case the untreated blood was held in the refrigerator for ninety-nine hours before injection, and it produced typical dengue. In several cases the virus was outside the body forty-eight hours, and in one seventy-two hours.

These authors have reviewed critically the work of Ashburn and Craig (1907) upon the filtrability of the virus. Their first series of experiments were not satisfactory, in that they resulted in only one apparently positive case and several negative results from the injection of the filtered blood. They repeated the work later. The technic used, as they give it themselves (pages 228 and 230), is as follows:

> The filter used was a small candle about six inches long with a wall one-eighth to one-sixteenth of an inch thick at the top, the inside diameter at the top being ca. one-third of an inch. The filter tapered slightly from top to bottom. It was marked "Chamberland *Sme.* Pasteur B. E. S. G. D. C. H. B. & Cie, Choisy-le-Roi, *BES* S. G. D. B." and stamped "Contrôle," and on the bottom "F." It is what is known as the Pasteur-Chamberland F. Filter, which is said to be a coarser grade than the B. type.
>
> For filtration, previously unused candles were fixed in bored rubber corks into the neck of flasks which had side tubes. The whole apparatus was sterilised by steam and cooled, and then attached by the side tube, which was plugged with cotton wool, to the rubber pipe leading to a water pump. The cork was covered with melted paraffin to be sure no leak occurred. The material to be filtered was run into the candle and

the pump turned on. When sufficient material had been obtained the plug was removed from the side tube, the end of this flamed and cooled, or wiped with alcohol, and the flask tilted and the filtered material run out into a sterile tube. Generally speaking the pressure at the Health Department Laboratory is not good and the later filtrations were done at the Royal North Shore Hospital Laboratory.

To show that the filters used did not admit the passage of ordinary bacteria, the practice was adopted of mixing organisms with the material to be filtered and testing for their presence by culture in the filtrate. All the cultures remained sterile.

Our results indicate that the dengue virus under certain conditions passes through the Pasteur-Chamberland F. Filter, which at the same time is preventing the passage of ordinary bacteria. The question now arises as to what is the cause of the failure in certain experiments.

Confining ourselves to the present series of tests, Case 19 may be rejected as a test as to the filtrability of the virus for reasons given above. In the negative cases, 5, 7, and 9, however, the virus was almost certainly present in the blood on withdrawal and in Cases 5 and 9 was actually shown to be present in the unfiltered blood from which the filtrate was made.

The sojourn outside the body less than 30 hours in any of the cases, is less than in two of our positive filtration cases and in several of our non-filtration positive cases, so that it is at first sight hard to find any ground for the failure of these cases. It is most unlikely that they were all immunes.

The three unsuccessful cases were obtained with filtered blood which before filtration contained a certain amount of solid material and the filtration was done in the Department's laboratory, and although we have no precise notes on the matter, the filtration was certainly slower than in the later cases, including three successful filtrations. Of the later cases, although in Case 13 filtration was also performed at the Department's laboratory, the filtration took place rapidly, the fluid being free from cellular material. In Cases 15, 17 and 19 filtration took place under better pressure conditions at the North Shore Hospital and filtration was more rapid.

We therefore conclude that the failure of certain of our cases was due to slow filtration and the plugging of the filter pores by solid material through which the fluid had to pass, and the successful cases were due to the more rapid filtration brought about by a higher water pressure and an absence of solid material.

Among the most remarkable results that have been reported concerning the transmission of dengue through injection of blood are those of Koizumi, Yamaguchi, and Tonomura (1918). They state that 5 cubic centimeters of blood from a patient on the third day of the disease was incubated ten minutes and centrifuged, and the clear serum injected. Infection occurred in five days twenty-two hours. They report that blood removed from patients up to the sixth day of the disease was infective, but that taken on the eighth day was not, although the patients into whom it was injected might have been naturally immune. They attempted to ascertain the minimum amount of the virus blood that would cause the disease in susceptible persons. The minimum amount they found to be infective was 0.00005 cubic centimeter. The average length of the incubation period was one hundred thirty hours. In two cases the virus blood was treated with three and four volumes of saline, kept at room temperature for five hours, and then injected into volunteers with positive results; when it was kept for five days in the ice chest, however, the result was negative.

They also made experiments upon the filtrability of the virus and had one successful result out of four; they state, however, that the other three men were later found to be immune. They add the comment concerning their experiments in mosquito transmission that the volunteers were not under observation and confinement before the experiment began, and for that reason they do not consider the results dependable. It is unfortunate that in the review of the paper, which is available to us, no information is given concerning the management of the volunteers who were injected with virus blood. Some of the results are so unusual one is inclined to wonder whether or not the other volunteers, especially those who received tiny fractions of a cubic centimeter of blood, were completely protected from outside infection.

Chandler and Rice (1923) injected blood intravenously into three volunteers; the dose administered was 1 cubic centimeter. One person was injected with blood taken from a patient forty-four hours after onset. The result in this case was negative, but the volunteer had been bitten previously by two batches of mosquitoes, with negative results. The presumption that he was immune seems justified. The two others were positive—in one case the blood was withdrawn four and a half hours after onset; in the other, about twenty-four hours after onset. The incubation period in the first case was five days nineteen hours; in the second case, five days three hours.

Blood counts.—Among the valuable additions to our knowledge of the clinical characteristics of dengue, recent research has brought forward the fact that in dengue there is a tendency toward leucopenia, and in individual cases the number of leucocytes may be exceedingly low. At the same time, there is a relative lymphocytosis.

THE CAUSATIVE AGENT

The etiology of dengue is the subject of a special section of this report, but in that section only the contributions to our knowledge which seem to be of suggestive value are discussed, and these were deliberately not considered from the standpoint of their historical interest. We now feel that we possess some knowledge concerning most of the important problems presented by dengue, but with regard to its causative agent we are still ignorant. Numerous careful and painstaking attempts have been made to identify and to isolate the causative microörganism, but so far the results have been negative.

According to Hirsch (1881), Charles (1873) described an organism in the blood. His work was done before technical procedures in bacteriology had been well developed. McLaughlin (1886), who worked at a time when bacteriological investigations into the etiology of disease were bringing such rich rewards, had to make use of methods with which only the most careful and fortunate workers were able to get clean results. The skin cocci frequently got into their blood cultures, and only the study of adequate controls prevented these and other contaminating bacteria from leading the investigators astray. McLaughlin was apparently the first to think the tiny round bodies seen in fresh and stained smears of the blood were the etiological agents of dengue. He thought these bodies, which measured one-twentieth to one-thirtieth the diameter of a red corpuscle, were spherical micrococci. In his cultures he found similar micrococci. His results were quite naturally taken seriously at the time; but, apart from the fact that some workers are still finding the little spherical bodies in dengue blood and taking them seriously, the work of McLaughlin is purely of historical interest.

Having convinced himself that the mosquito was the means by which the disease was carried from one person to another, Graham (1904) came to the conclusion that the causative microorganism must be located in the circulating blood. In the hope of finding it, he examined over one hundred cases of dengue. Nowhere, however, does he say anything about having examined the blood of persons who did not have dengue. Sometimes the blood was taken from the finger tip, but frequently it was also drawn from the brightest spots of the eruptions. He states that he found in the red blood corpuscles an organism with amœboïd movement in many ways resembling *Plasmodium malariae.* He says of it (page 212):

> It is evidently a protozoon, but without pigment, and in this it differs from the *Plasmodium malariae.* It is true that Marchiafava and Celli have discovered and carefully described an unpigmented quotidian parasite, but it differs so in its life-story that it need not for a moment be confounded with this. I know of no blood parasite which resembles it so much as the *Pirosoma bigeminum* (*Boophilus bovis*). It, like the *Pirosoma,* is mostly ovoid in form and very frequently shows a sharply pointed extremity as seen in the parasite of Texas fever. Both are unpigmented forms, but the haematozoon of the dengue has not the divided form which has given the name *bigeminum* to the *Pirosoma.*

In the summer of 1902, Graham began a series of experiments in an attempt to follow the parasite through the definite phases of its life into the body of the mosquito. "These experiments were done *almost wholly* [8] with the *Culex fatigans,* which I found in every way, by reason of its habits, verocity [sic], etc., a much more facile insect with which to experiment." Mosquitoes were permitted to bite dengue patients and then, every day subsequently, one of these was killed and carefully examined. The blood of the stomach was found to contain the parasites and the changes they underwent in the metamorphosis were observed to be similar to those found in blood drawn directly from patients, although it appeared to him that development in the stomach of the mosquito was more rapid than in the patient. He never failed in any of the cases to find the parasite up to the fifth day after the mosquito had sucked the blood, and it seemed to him that the parasite was detected with more ease and certainty in the blood of the stomach of the mosquito than in that drawn directly from the vein of the patient. Cases in which the mosquitoes drew the blood early in the attack took more time to develop full-grown spores than did those in which the blood was sucked later.

These observations were made with such apparent care that they necessarily attracted widespread attention, and numerous workers spent many hours investigating the validity of Graham's findings. It was not until 1910 that anybody else found it possible to agree with Graham, and such agreement as was furnished at that time came from his assistant, Nagib Ardati (1910). Ardati says that about sixty hours after the beginning of an attack the parasite, which he calls *Haemamoeba denguii,* seems to reach its maximum size and begins to migrate out of the erythrocytes, usually rupturing them. From this time on, there are many extracorpuscular parasites in addition to those which are intracorpuscular; "the extracorpuscular parasites look like a group of fine granules, arranged more or less in circles, sometimes two or three of them lie side by side, each of them resembling a group of blood platelets. In the leucocytes are often found small granules which seems to show that the parasites were taken up by the white corpuscles."

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[8] Italics ours.

It is curious, considering the number of careful workers who failed to confirm Graham's results, and who called attention clearly to the fact that the bodies observed by him were probably artefacts and occurred just as frequently in the blood of normal persons, that this assistant of Graham's makes no statement in his report that he had examined the blood of normal people, or of persons suffering with other diseases. Unfortunately this worker is not the last who has based his report upon such lack of controls.

Ashburn and Craig (1907) state that Klein investigated very carefully the report of the presence of a short bacillus in the blood of dengue cases; his conclusion was that there was not sufficient evidence to associate this organism consistently with the disease; that Wright was unable to demonstrate that any organism was concerned in the etiology of dengue; and that similar reports were made by Crookshank and MacFadyen.

Eberle (1904) examined the blood of *Culex fatigans* fed on dengue cases and thought he found a plasmamœbic organism. He examined both fresh blood and stained smears. The bodies he reported were undoubtedly vacuoles and artefacts. The pictures he makes do not look very much unlike those shown by Graham.

Carpenter and Sutton (1905) examined both fresh and stained specimens of blood from cases of dengue occurring upon the Isthmus of Panama. They used various blood stains as well as bacterial stains, without being able to demonstrate either McLaughlin's micrococcus or any other microörganism in the blood. They found the nonpigmented bodies of Graham in unstained specimens, but not in stained ones. They also found Graham's *Haemamoeba denguii* in the blood of patients suffering with other diseases. In mosquitoes that had bitten dengue patients they found nothing resembling parasites.

Kieweit de Jonge and de Haan (1905) made most careful examinations of the blood of patients in Java, but without demonstrating the presence of any parasite.

Guiteras (1906) made careful investigations during the epidemic in Havana in 1905. He examined a large series of specimens of blood taken during all stages of the disease. The blood was stained by various methods, but no structure resembling a parasite could be found, and he felt that Graham was mistaken in regarding his organism as a parasite.

Agramonte (1906) likewise studied the blood of dengue patients in Havana, and found it impossible to find any parasite in the blood. Stitt (1906) likewise made most careful examinations of cases which occurred at Cavite, Philippine Islands. He also was unable to demonstrate the presence of any organism, either protozoal or bacterial, in the blood of his patients.

Reiche (1906) found in nearly every case he examined very small round translucent bodies with clearly defined edges. They were about 2 microns in diameter. They were very motile and easily found with a $\frac{1}{6}$ objective. They were not in the erythrocytes, but often on them. Reiche apparently considered it not worth while to examine the blood of normal persons or of those suffering with other conditions.

Ashburn and Craig (1907) made careful examinations of both fresh and stained preparations of blood. They were familiar with the failure of numerous observers to confirm the presence of McLaughlin's or Graham's microöganisms, and also the negative results that had been met with in the attempt to find parasites. Nevertheless, for the sake of the completeness of their work, they felt constrained to investigate the possibility of identifying the etiologic agent of dengue. The blood was examined every day during the disease, especially the first two days, and during the terminal rise in temperature. They employed various staining methods, including Wright's stain and the methods used for demonstrating *Treponema pallidum*, "the latter methods were used very carefully and in numerous cases, as at the time we began our work, we were greatly inclined to believe that the organism concerned in the etiology of dengue might belong to the spirochaete. We have not been able to confirm the results of McLaughlin or Graham, nor have we been able to demonstrate any organism in the blood of dengue patients which we can consider an etiological factor."

J. C. D. Allen (1906), in his paper on the epidemic studied by him, stated that only a limited number of patients produced any sputum; however, when sputum was available and it was examined, it revealed at the height of the fever the invariable presence of a spirochaete. Often long search was required to find it. On the other hand, after the subsidence of the fever, none could be found. There is no evidence that Allen looked for mouth spirochaetes in persons who did not have dengue.

In the course of their attempts to produce dengue in monkeys, Lavinder and Francis (1914) planted various quantities of blood from all their cases in freshly boiled gluclose broth fermentation tubes. Some of these were kept at room temperature, and some at 37° C., for several days. Even with the most careful technic, growth appeared in some of the tubes, but they felt that such growths could safely be considered merely contamination.

Couvy (1914, 1921), in Beirut, found short slender spirochætes in the blood of patients with dengue. These spirochætes were very slender, having two or three turns and fine extremities. In the cases examined which gave positive results, the blood was taken from the patients one or two hours before the beginning of the fever. Spirochætes have never been seen during the course of fever, nor after defervescence.

Cleland, Bradley, and McDonald (1919) regard the bodies described by Graham as artefacts. In discussing the distribution of the virus in the blood they say that, although the virus has been shown to be filtrable (thus showing that at some stage it must be very small), the possibility of an endocorpuscular phase of a microscopically visible size is, of course, not excluded, "although we have examined blood from dengue cases carefully a number of times with unstained preparations, and with the ultramicroscope, we have not found any evidence of a visible virus."

Polymorphous organisms are reported to have been found by Holt (1922) in the blood of patients and inoculated animals.

The careful work of Chandler and Rice (1923), done so recently, makes their observations of more than passing interest. Their efforts were directed chiefly to the demonstration of a *Leptospira*. Their failure to find any such organism has led them to draw deductions (pages 240 and 241) which we believe should be quoted here.

> In view of the consistently negative results which we have obtained in our search for a Leptospira-like organism by means of dark field

examinations of fresh blood from patients and from inoculated animals, and by means of cultural methods which are successful with known species of Leptospira, we consider it improbable that the causative organism is of spirochetal nature. This conclusion is strengthened by a number of other considerations. Guinea-pigs are readily susceptible to the known kinds of Leptospira (*L. icterohemorrhagiae, L. icteroides, L. hebdomadis*), but are apparently quite immune to the virus of dengue. Moreover, in all the known Leptospira diseases (yellow fever, infectious jaundice, Japanese seven-day fever), the liver and kidneys are the organs most uniformly and injuriously attacked, resulting in jaundice and albuminuria, with excretion of the parasites with the urine, but in dengue neither the liver nor kidneys are ordinarily attacked in uncomplicated cases. The fact that Cleland, Bradley and MacDonald were able to produce the disease by inoculation of blood which had been kept in an ice chest for as long as seven days also argues against the organism being a Leptospira since the latter is extremely short-lived after the death of the host, especially so in the case of *Leptospira icteroides* of yellow fever. The *Leptospira* theory is based solely on the supposed relationship between dengue and yellow fever, and as we have already shown, this relationship is not as close as commonly assumed. The fact that *Aëdes aegypti* is the principal if not the only transmitter of dengue as well as of yellow fever can hardly be construed as an important argument in favor of the close relationship of the organisms involved in the two diseases. As Noguchi has pointed out, *Aëdes aegypti* probably owes its distinction as the sole carrier of yellow fever to the fact that its gut offers the two conditions necessary for the growth of the causative organism; namely, sterility so far as bacteria are concerned, and the presence of at least minute quantities of blood or serum for food. That these same factors might favor the growth of other organisms, not necessarily Leptospira, is by no means improbable. It is of interest to note that administration of arsphenamin did not have any effect in warding off an attack of dengue. Numerous cases developed in patients who were under intensive treatment for syphilis.

Finally, we have to note the recent work of Duval and Harris (1924). This work is discussed in the section on etiology and needs no further comment here.

One member of this board found a spirochæte-like form in the blood of several dengue patients, but careful work with the blood of controls demonstrated that this form has nothing to do with the etiology of dengue.

Animal transmission.—In the older literature, there are numerous references to the occurrence of a similar disease among domestic animals during epidemics of dengue. This was thought by some observers to mean that these animals were actually infected by the same virus. In view of the results of careful laboratory work which have been almost constantly negative, there can be no doubt that the animals were suffering from some other infection.

Lavinder and Francis (1914) attempted to reproduce dengue in the monkey by the inoculation of defibrinated blood. The following is quoted from their report (pages 343 and 346):

> To this end we secured blood from the arm vein of cases of dengue in various stages of the disease, and after defibrination, this was injected intravenously and subcutaneously into monkeys, all as shown in the table given below. The blood was drawn at the residence of the patient with a glass syringe, expelled into a large tube, defibrinated by carefully stirring with a sterile glass rod, brought at once to the laboratory and put in the ice chest until used. For use it was filtered through gauze to get rid of clots. All the monkeys were fresh, healthy, young animals.

TABLE 1.—*Showing cases of dengue and animals inoculated.*

Cases	Quantity blood injected	Remarks
E.—4-5th day illness, in eruption	10 c.c. intravenously; 3 c.c. intravenously.	Rhesus 9; rhesus 14; same blood kept 24 hours in ice chest.
R.—3-4th day illness	8 c.c. intravenously	Rhesus 10.
McA.—2-3d day illness	6 c.c. intravenously	Rhesus 16.
S.—4th day illness, in eruption (?)	6.5 c.c. intravenously	Rhesus 23.
W.—2d day illness	5 c.c. intravenously; 1 c.c. subcutaneously.	Rhesus 25.
F.—5th day illness, in eruption, white blood cells, 3,000.	5 c.c. intravenously; 1 c.c. subcutaneously.	Rhesus 24.
R2.—3-4th day illness, in eruption; white blood cells, 5,900.	6 c.c. intravenously	Rhesus 71.
V.—3d day illness, in eruption; white blood cells 8,200.	10 c.c. intravenously	Rhesus 73.

> To summarize, it will be seen from the above that we injected intravenously and subcutaneously from 3 to 10 c.c. of defibrinated blood from each of eight cases of dengue into nine rhesus monkeys. The blood was used within two to six hours after withdrawal in all cases but one, where it was kept twenty-four hours in the ice chest. The cases from which the blood was drawn were all carefully selected and in several of them the diagnosis was confirmed by the presence of the characteristic eruption at the time of bleeding. All the animals were carefully examined each day for illness of any kind and for skin eruptions for a period of two weeks. During the same period the temperatures of all were recorded twice daily. They all remained well throughout this period of time and showed no significant deviations of temperature, nor was there any significant skin eruption observed in any of them.
>
> From our work we feel justified in concluding that if dengue is conveyable to the rhesus monkey by intravenous or subcutaneous inoculation of defibrinated blood, these animals do not show the disease by changes in temperature, appearance of skin eruption or any of the ordinary symptoms of illness; further that it is doubtful whether they show any definite and characteristic change in the white blood cell count, but the results obtained by us are perhaps sufficiently suggestive to invite further effort.

Kraus (1916) attempted to infect guinea pigs by the injection of blood from patients. The guinea pigs during the eight days of observation subsequent to their infection showed neither fever nor any other symptom indicative of infection.

The results of Koizumi, Yamaguchi, and Tonomura (1917) have already been commented upon. These investigators found that small amounts of defibrinated blood from dengue patients gave negative results when injected into dogs, rabbits, white mice, and long-tailed Formosan monkeys. Guinea pigs, however, died seven to thirty-six days after injection. Blood from the hearts of guinea pigs that had been inoculated fifteen days previously caused the death of other guinea pigs after five to nineteen days when injected intraperitoneally or subcutaneously; if, however, the guinea-pig blood was injected intravenously, death did not occur in the second series until the twenty-eighth or thirty-fourth day. The infection could in no case be transferred through a third series.

Cleland, Bradley, and McDonald (1919) attempted to transmit the virus through a guinea pig to a human being. They took blood from a patient twenty-two hours after onset, and in the afternoon of the same day injected 0.25 cubic centimeter subcutaneously into a guinea pig. The animal remained well and in the morning of the eighth day thereafter it was bled to death. On the same day, 2 cubic centimeters of the whole blood of the guinea pig were injected into a volunteer. The result was negative. They conclude that there is no evidence of the survival of dengue virus after seven and a half days in the guinea pig, nor of its multiplication in this animal. They likewise injected guinea pigs and rabbits intraperitoneally and subcutaneously without results. They made sections from the organs of some of these animals, after staining by both iron hæmatoxylin and eosin and by Levaditi's method. The tissues so examined showed no abnormalities. Levaditi's method was used in the hope of demonstrating possible spirochæte-like organisms. This experiment was undertaken because of the results obtained with epidemic jaundice in France.

Holt (1923) was unable to produce symptoms in guinea pigs and rabbits by the injection of blood of dengue cases.

Armstrong (1923) attempted to produce dengue in guinea pigs, rabbits, white rats, and rhesus monkeys, but with negative results. He says:

> Animals were injected with blood taken at various stages of the disease (fifth hour to convalescence). The animals were observed for from eight to thirty days in various cases. In no instance was the behavior of the injected animals different from that of the controls. Blood counts were

made on monkeys, rabbits, and rats, but no variations deemed significant were noted. In the case of guinea pigs, however, the results were complicated by an epidemic of broncho-pneumonia which broke out among them after about fifteen days. Controls and infected animals were alike attacked.

Armstrong states that the tissues have not been examined, and that the experiments will be made the subject of a more-detailed report.

The results of animal inoculations made by Chandler and Rice (1923, pages 236 to 238), as given by themselves, are as follows:

> Animal inoculations were tried with guinea-pigs, white mice, and a rhesus monkey. Fourteen guinea-pigs were inoculated intraperitoneally with from 2 to 3 cc. of whole blood inmediately after it was drawn from the patient, 2 guinea-pigs being inoculated simultaneously from each of 7 patients, the blood being taken on various days of the disease from the first to the fifth. In all cases but 1 the guinea-pigs remained perfectly well during the period of observation (three to four weeks), and showed no significant rise in temperature. One guinea-pig (No. 3) inoculated from a patient twenty-four hours after onset, appeared slightly sluggish and indisposed on the fifth day, with a temperature of 102.2° F., which, however, he had registered before inoculation. This pig was killed and autopsied, and 2 other pigs inoculated intraperitoneally with its blood, one with 4 cc., the other with 2 cc. Neither of these pigs showed any signs of illness, or any lesions, nor were any pathological conditions found in pig 3. Inasmuch as Noguchi (1919) found that yellow fever did not always produce a typical disease in guinea-pigs until the first or second subinoculation, we drew blood from the heart of another guinea-pig (No. 6) on the fifth day after inoculation with a patient's blood taken on the second day of the disease, and inoculated 1 cc. intraperitoneally into pigs 19 and 20. On the fifth day we drew heart's blood from pig 19 and inoculated it in like manner into pigs 17 and 18, but in all cases with negative results. The blood from pigs 3, 6, 18, and 19 was examined under a dark field microscope, but with negative results.
>
> From another patient 2 guinea-pigs were inoculated in a somewhat different manner. One cubic centimeter of a patient's blood, drawn on the second day of the disease, was diluted with 1 cc. of heart's blood from a guinea-pig by immediately plunging the needle containing the patient's blood into the pig's heart and withdrawing enough blood to make 2 cc. In one of these pigs (unanæsthetized) 1 cc. of the mixture was reinjected into the heart, the remaining 1 cc. being inoculated intraperitoneally. In the other pig, which was under ether anæsthesia, the same procedure was followed except that the intraperitoneal injection was not made, the second 1 cc. of blood being discarded. Both of these pigs remained well, but thirty-one days later 6 cc. of blood was withdrawn from each of them to lower their vitality (Japanese investigators found that guinea-pigs succumbed in from seven to thirty-six days), but this had no effect.
>
> It occurred to us that it might be possible to produce infection in guinea-pigs by using a very small quantity of blood, thus carrying over

fewer antibodies, if these were present. Noguchi (1919) apparently found that such antibodies were a factor in animal inoculations and it was evident that very small quantities of blood were sufficient to cause infection. Koizumi, Yamaguchi, and Tonomura (1917) state that 0.00005 mil. was found to be infective for man. To test this point we withdrew 0.1 cc. of blood from a patient in the second day of the disease and diluted it with a guinea-pig's heart's blood to 1 cc. and then re-injected 0.02 cc. of the mixture into the heart, thus inoculating 0.002 cc. of patient's blood if the mixture had been perfect, but in all probability the pig received considerably less than this of the patient's blood. This pig also remained well. Furthermore, 65 guinea-pigs, of all ages, were housed in an outdoor pen during the entire summer and fall, where yellow fever mosquitoes swarmed. Only one became ill, and three days later died from a respiratory infection. These pigs were freely bitten, even tormented, by the mosquitoes, and the percentage of dengue in that district was high.

These results with guinea-pigs are in harmony with results obtained by Cleland, Bradley, and McDonald (1919) in Australia, who inoculated a non-immune human being with 2 cc. of blood drawn from a guinea-pig on the seventh day after inoculation with dengue blood, with negative results. There is, however, no corroboration of the work of Koizumi, Yamaguchi, and Tonomura (1917) who state that small amounts of defibrinated dengue blood were lethal to guinea-pigs in from seven to thirty-six days, and that blood taken from pigs inoculated fifteen days previously was lethal to other pigs in from five to nineteen days when inoculated intraperitoneally or subcutaneously, and in from twenty-eight to thirty-four days if inoculated intravenously, while the third transfers were negative. It is significant that in an earlier paper Yamaguchi, Ditsumi, and Tonomura (1916) state that inoculations into guinea-pigs were without results. We conclude, therefore, that guinea-pigs are not susceptible to dengue organisms.

We inoculated 6 white mice with about 0.5 cc. each of fresh citrated dengue blood taken on the third day of the disease. With the exception of one mouse which died during the inoculation all of the mice remained perfectly well. Rats were not available for experimental work.

We inoculated a young rhesus monkey with about 5 cc. of citrated dengue blood, drawn on the third day of the disease, about two hours after the blood was taken. Although the monkey, according to his keeper, appeared to be chilly and unusually morose on the third and fourth days after inoculation, he showed no significant rise in temperature. On the fifth day he showed a fine papular rash on the chin and throat, which, however, we do not think was a manifestation of dengue. No other symptoms were observed and we are inclined to think that the inoculation had negative results, as did those of Lavinder and Francis (1914); at any rate, it was very doubtfully positive.

Harris and Duval (1924) reported studies made upon a group of guinea pigs. The results were complicated by the existence of infection with one of the paratyphoid group of microörganisms. Their results are sufficiently commented upon in the section on etiology.

We have investigated the possibility that the virus may remain or multiply in the blood of hogs. Four young animals were secured and held in quarantine for at least two weeks; blood counts were made frequently during this period. Two were injected intravenously with the virus blood from two early cases of experimentally produced dengue. Each hog received about 10 cubic centimeters of virus blood intravenously. One week later these two hogs were bled and the blood was mixed and injected immediately into the other two hogs, these likewise each receiving about 10 cubic centimeters. The latter two animals were bled one week after their injection, and the blood was brought to Manila from the serum laboratory of the Bureau of Science at Alabang, and 0.5 cubic centimeter was injected into two susceptible volunteers. Neither of these showed the slightest symptoms of dengue. Blood counts made subsequent to the injections into the hogs showed no significant changes.

SUMMARY AND CONCLUSIONS

1. Yellow fever and dengue, in the mechanism of their transmission, are practically identical, and the transmitting agent for both is *Aëdes aegypti.* These facts suggest that the history of dengue is likewise closely associated with the history of yellow fever. If this should be true, a study of the history of yellow fever might reveal evidence of the existence of dengue in earlier times in regions where yellow fever is known to have prevailed.
2. A search of the sources available has shown that yellow fever existed in tropical America prior to the first voyage of Columbus, and early reports of the epidemics that occurred in yellow fever areas indicate that dengue did exist at an early period, but was not always clearly differentiated from the more serious disease to which it is related.
3. As a result of our historical research we have formulated the hypothesis that dengue, as well as yellow fever, originated in tropical America.
4. The earliest epidemics of dengue of which we have accurate reports occurred in widely separated parts of the globe—Java, 1779; Cairo, Egypt, 1779; and Philadelphia, United States, 1780.
5. The account of Benjamin Rush, describing the 1780 epidemic in Philadelphia, is so clear there can be no doubt as to the identity of the disease. His description is lucid and corresponds closely with the symptoms of dengue as we have seen

them in Manila. There is a possibility that the epidemics in Java and in Egypt were not dengue.

6. Following these reports, epidemics of dengue were studied in all parts of the tropical world, and also in cooler climates to which the disease and the mosquitoes had been transported.

7. The studies of epidemics established the symptomatology and revealed the fact that relapses in dengue are not uncommon; recurrences are comparatively frequent, and a certain number of persons suffer several attacks. In addition, from evidence accumulated, the rapidity of the spread of dengue is possibly second only to that of influenza.

8. Although mosquitoes had been suspected as the transmitting agents of dengue, it remained for Graham to demonstrate that these insects actually do transmit the infection.

9. The work of Ashburn and Craig, of Cleland, Bradley, and McDonald, and of Chandler and Rice added to our information concerning mosquito transmission. The last two groups of authors suggested that the evidence indicated that *Aëdes aegypti* is the transmitting agent.

10. The work of this board has shown conclusively that *Aëdes aegypti* is the transmitter of dengue, and that *Culex quinquefasciatus* is not.

11. Ashburn and Craig were the first to show that the dengue virus is contained in the circulating blood, and they were the first investigators to demonstrate that the cause of dengue is a filtrable virus.

12. Cleland, Bradley, and McDonald made important studies upon the distribution of the virus in the blood showing that it is present in all elements of the blood, although they suspect that it is attached merely to the erythrocytes; they found that the virus was present in the blood up to ninety-nine hours after onset, and that the drawn blood retained its infectivity for seven days if kept in the ice chest.

13. Carpenter and Sutton first called attention to the leucopenia, which is a fairly constant symptom of dengue.

14. All attempts to transmit dengue to the lower animals have resulted in failure to obtain clear-cut indications of infection.

15. The work of this board has shown that the *Aëdes* mosquitoes transmit dengue; that they become infective by biting a patient during a period beginning a few hours before the first symptoms and up to the end of the second day. Infec-

tion of the mosquitoes is more or less uncertain after that time. Having taken the blood of the patient, the mosquito becomes infective only after the tenth day and retains its infectivity probably throughout the rest of its life.

16. Clinical observations with regard to the uncertainty of immunity to dengue has been experimentally confirmed. Natives of areas in which dengue is endemic are immune, but their immunity is believed to be acquired and not natural.

17. Carefully controlled studies to identify and attempts to isolate the causative microörganism of dengue have so far not been successful.

TRANSMISSION OF DENGUE BY MOSQUITOES, I

INTRODUCTION

Before beginning the experimental work on transmission of dengue by mosquitoes, the results of which are to be presented in this report, it was necessary to plan and prepare the equipment and to provide means by which pertinent materials of all kinds would be available when required. After deciding upon the scope of the investigations to be undertaken, detailed consideration was given to those matters which concerned directly the mosquitoes to be used in the studies on the one hand, and the human subjects on the other. For the mosquitoes, decision was made with regard to the species to be experimented with, the methods of their procurement, and specifications for cages and other containers to be used for their breeding and for maintaining and storing the adults. For the volunteer human subjects, consideration was given to the method of procurement and the qualifications to be met by them. Finally, a mosquito-proof ward for the isolation of experimental subjects was constructed and strict rules laid down for its administration.

In Part I of this section the above-mentioned features of the investigation are considered; the actual experimental work and conclusions drawn therefrom are dicussed in Part II.

The plan for every experiment was first submitted to all members of the board for their constructive criticism or for further elaboration, and the experiment was finally initiated after agreement on the method of procedure.

Much time was consumed in the work, due primarily to the fact that the results would be more clear-cut, it was felt, if only one phase of the problem were considered at a time. Incidentally, a large number of suitable volunteers could not have been obtained at one time, unlimited ward space was not available, and simultaneous concentration on several phases of the problem would have necessitated the use of enormous numbers of mosquitoes.

The actual experimental work with volunteers was not begun until August, 1924, and was completed in March, 1925, a preliminary period of approximately four months having been required for the development of efficient mosquito-breeding methods and for the completion of preliminary arrangements.

The personnel engaged directly in the transmission work consisted of one member of the board, part time (Siler), in general charge of investigations; one technical assistant, full time (Rhodes), in general charge of mosquito breeding and handling of experimental biting; one technical assistant, full time (Reyes), in charge of breeding *Aëdes aegypti* Linnæus; one technical assistant, full time (Tanga), in charge of breeding *Culex quinquefasciatus* Say; one secretary, part time, in charge of records; one member, Army Nurse Corps (Second Lieutenants Nichols and Carter, successively), in charge of the experimental ward; two members, Medical Department Detachment, Sternberg General Hospital, ward assistants, experimental ward (day duty); one member, Medical Department Detachment, Sternberg General Hospital, ward master, experimental ward (night duty).

Of the seven individuals engaged in the investigation (excluding the officer in charge and the secretary), three were required for laboratory duties and four for duty in the experimental ward. In conducting investigations of a like nature provision should be made for at least one technical assistant in excess of actual requirements, to meet temporary emergencies arising through illness or other cause.

The experimental ward at Sternberg General Hospital is approximately 2 kilometers distant from our laboratories at the Bureau of Science, and the transportation used was one calesa (two-wheeled, horse-drawn, passenger vehicle). Privately owned transportation was used to a very considerable extent. In organizing a similar investigation it would be necessary to make provision for one motor-driven passenger car.

At the peak of our experimental work, we used four breeding and reserve cages, sixteen small breeding cages, forty-two biting cages, seventy-two breeding jars, and sixteen breeding trays. Descriptions of this equipment will be found in succeeding pages.

The sum of 1,000 pesos[9] more than sufficed to cover the cost of incidentals of this nature over a period of twelve months.

Each individual who volunteered for the transmission experiments was allowed a gratuity of 25 pesos.

The Military Service proved to be an ideal organization for the conduct of these investigations for the following reasons: A research board existed for the prosecution of the work; ward facilities were available in a military hospital for the prosecution of the experiments with human subjects; and commanders of all grades coöperated with us whole-heartedly in securing volunteers from military commands.

SCOPE OF EXPERIMENTAL WORK

The initial basic transmission experiments were planned with two definite aims in view; namely, confirmation of previous experiments relative to the transmission of dengue fever by *Aëdes* (*Stegomyia*) *aegypti* Linnæus, and incrimination or elimination of *Culex quinquefasciatus* Say (*C. fatigans* Wiedemann) as a transmitting agent.

These two points having been settled to the satisfaction of the board, it was possible to initiate a further series of experiments having for their purpose the discovery of the exact mechanism of transmission of dengue by mosquitoes.

CAGES AND OTHER EQUIPMENT USED IN BREEDING AND STORING STOCKS OF MOSQUITOES

Breeding containers for larvæ and quality of water used therein.—After working with several kinds of breeding containers, the type finally selected for *Aëdes* breeding was a screw-top glass fruit jar of 1,000 cubic centimeters capacity (Mason type), and for *Culex* breeding, a rectangular pan (photographic developing tray).

Boiled, distilled, and tap water were experimented with as stock breeding solutions, and tap water gave the best results.

The water supply for Manila is obtained from the headwaters of Mariquina River, near Montalban, approximately 25 kilometers from the city. Chemically, the quality of the water is good, as is evident from the analyses incorporated in the Appendix to this series of reports.

The water is somewhat hard, the mineral content is low, and usually it has slight turbidity (clay and silt). The water is chlorinated, and the report on chlorine content (Table 1) is typical of analyses usually reported.

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[9] One peso is equivalent to 50 cents United States currency.

TABLE 1.—*Examination*[a] *of Manila water*[b] *for free chlorine.*

[Date of examination, January 9, 1924.]

Time.	Chlorine, parts per million.
8.30 a. m.	0.4
12.00 m.	0.2
2.00 p. m.	0.3
4.00 p. m.	0.35

[a] Analysis by E. Taylor, chemist, Bureau of Science.

[b] Turbidity, 5.0 (as parts per million of silica).

Varying amounts of water were used, and the optimum was found to be 250 cubic centimeters, giving a depth of 38 millimeters. It was found, however, that losses by evaporation gave rise to a somewhat unfavorable environment, and as a matter of routine the water was kept at a constant level (38 millimeters) by replenishment every two or three days.

The breeding jar for *Aëdes* is illustrated in Plate 1, fig. 1.

A large circular opening was cut in the metal top of the jar and over this was placed fine netting strapped on with adhesive plaster (see Plate 1, fig. 1). This modification assured free circulation of air. As the jar was made of clear glass, an unobstructed view of its contents was permitted.

For *Culex* mass breeding, if large numbers of insects are required, as was the case in these investigations, any kind of basin that will hold an ample supply of water will answer the purpose, provided it is white so as to facilitate observation of the larvæ, and provided the depth of the breeding solution is at least 38 millimeters.

In our *Culex* breeding work, we used a white enamel, rectangular, photographic developing tray, 30.4 by 35.5 by 5.08 centimeters, illustrated in Plate 1, fig. 2.

Into this tray were placed 2,000 cubic centimeters of water, and a constant level was maintained. The area of surface exposed to evaporation is large, and it was found to be particularly important to keep the water level constant by additions at frequent intervals (every two or three days). Close attention to this point impressed us as being of considerably greater importance in *Culex* breeding than was the case with *Aëdes.*

Breeding cages.—Two types of breeding cage were used, one for the storage of adult male and female *Aëdes* for egg-laying purposes, and the other for the collection of adult mosquitoes as they emerged from the pupa cases.

The type of storage cage used for egg-laying purposes is shown in Plate 2, figs. 1 and 2. This cage was 28 inches long, 22 inches wide, and 22.5 inches high (approximately 71 by 56 by 57 centimeters). The framework and floor were of wood. One end was constructed of wood, with a sliding wooden door (35.5 centimeters long by 31.75 wide) to permit the introduction of a cage containing a rabbit or a guinea pig. (Blood feeding on laboratory animals was found to be unsatisfactory in our work, and this door was permanently closed.) The front was clear glass (to permit unobstructed observation) and the back, top, and remaining end were covered with fine netting. Near the bottom in the back of the cage was an opening (see Plate 2, fig. 2), 6 inches (15.24 centimeters) square, to which was attached a sleeve made of netting and through this sleeve jars for egg laying and for mosquitoes were introduced and withdrawn and food was supplied. The inside of the cage was painted white to facilitate observation of the adults.

The type of cage used for the collection of adults as they emerged is shown in Plate 3, fig. 1. These cages were 17.5 inches long, 14.5 wide, and 13 high (approximately 45 by 37 by 33 centimeters). The framework was of wood, with a floor of light wood or fiber board. The covering was a fine netting and the opening into the cage was through a net sleeve. The inside of the framework was painted white.

Cages used for experimental biting.—These cages are illustrated in Plate 3, fig. 2. They were 20 inches long, 12 inches wide, and 12 inches high (approximately 50 by 31 by 31 centimeters). The frame was made of light-weight wood painted white, to facilitate counting the lots of mosquitoes. It was covered with closely woven netting, and access to the interior was through a sleeve of net, 12 inches in length (about 31 centimeters). The sleeve was closed with a draw string. The netting was stretched smoothly over the frame and held in place with cotton tape tacked to the frame (see Plate 3, fig. 2). If this detail in construction is neglected there are likely to be small spaces between the netting and the framework into which mosquitoes can make their way, become entangled in the mesh, and die or inadvertently be crushed.

Storage cages for reserve stocks of mosquitoes.—The type of cage used for this purpose was identical with that used for the storage of male and female *Aëdes* during the egg-laying period (see Plate 2, figs. 1 and 2).

Type of netting used to cover cages.—Great difficulty was experienced in securing a suitable type of netting for use in covering the various types of cages. We first used a bobbinet (approximately 12 meshes to the inch), approximating in quality that specified for mosquito nets used by the Medical Department of the Army in its hospitals. The stocks of such net in Manila were small, and were quickly exhausted. It then became necessary to resort to the next best quality available. Experiments in shrinking the material gave us a net with appoximately 12 meshes to the inch.

Shrunken netting was used on a number of cages but proved to be unsatisfactory for our purposes. Several lots of infected mosquitoes stored in these cages began to be checked short and the two persons (Siler and Rhodes) most closely and most intimately in contact with them became infected with dengue fever. There is no doubt in our own minds that the infections were acquired in the laboratory and were due to infected mosquitoes that had escaped through the netting.

It was necessary to secure a more finely meshed netting immediately, and the kind used in making women's dresses was selected (see Plate 4, figs. 1 and 2). It is about 91.5 centimeters in width, has approximately 16 meshes to the inch, and the retail price in Manila was 2 pesos a meter. This netting was placed on all cages (reserve, breeding, biting, etc.) used by us, and subsequent to that time no losses by escape through the net occurred, provided the mosquitoes were approximately normal in size.

In the late stages of the work (February, 1925) some difficulty was experienced for a short time in breeding out normal-sized adults, and occasionally, when it became necessary to use rather small mosquitoes, we checked shortages in a few lots. The small mosquitoes may have escaped through even this fine mesh.

Slight defects were found in all lots of cotton netting, and before newly constructed cages were put into use they were minutely inspected and openings closed with small squares of adhesive plaster. The best quality of the finer-meshed bobbinet, used commercially in the manufacture of mosquito nets, will prevent the escape of *Culex quinquefasciatus* and large-sized *Aëdes aegypti;* but, in the conduct of experimental work in mosquito transmission, we consider it essential that very fine netting, approximately 16 to 20 meshes to the inch, be used in the construction of all types of cages.

SPECIES OF MOSQUITOES SELECTED FOR TRANSMISSION EXPERIMENTS AND METHOD OF THEIR PROCUREMENT

Species of mosquitoes selected.—A review of the literature and the epidemiological observations made by ourselves and others in the Philippines convinced us that here only two species of mosquitoes, *Aëdes* (*Stegomyia*) *aegypti* Linnæus and *Culex quinquefaciatus* Say (*C. fatigans*), are worthy of consideration as transmitting agents. Our opinion was based on the following considerations:

The geographic distribution of dengue throughout the world coincides in general with the natural habitat of *A. aegypti*, and in areas of dengue prevalence *C. quinquefasciatus* is found in abundance.

Cleland and Bradley of Australia have pointed out (1918) that southern Australia is not the natural habitat of *Aëdes aegypti* and that dengue fever never prevails there in endemic or epidemic proportions, whereas in the northern parts of the Australian continent, which is the natural habitat of *A. aegypti*, frequent epidemics of dengue do occur. They state further that normally *Culex quinquefasciatus* is found throughout Australia.

Chandler and Rice have observed (1923) that the very widespread epidemics of dengue in Texas in 1922 coincided with the unusual prevalence of *Aëdes aegypti* mosquitoes throughout the area of epidemicity.

It is well known that throughout the Philippine Islands the two species of mosquitoes most commonly noted in human habitations are *Aëdes aegypti* and *Culex quinquefasciatus*. This statement applies particularly to the City of Manila where, for a number of reasons, dengue fever prevails much more extensively than elsewhere in the Islands.

Mr. W. Schultze, entomologist of the Bureau of Science, the members of our board, and other individuals competent to judge, have observed on innumerable occasions that the only two species of mosquitoes constantly present in houses in Manila, throughout the dengue season, are *Aëdes aegypti* and *Culex quinquefasciatus*.

During the 1924 dengue season, in coöperation with Maj. H. F. Hazlett, commanding the Second Battalion, Thirty-first Infantry, Manila, mosquito-catching details were organized, and mosquitoes were caught daily in the barracks of each company of his battalion. The work was carried out during the months of August and September at the height of the dengue-fever season, when dengue was occurring frequently in the personnel of the battalion. The experiment was initiated as a preventive measure and as *Culex quinquefasciatus* had not, at that time, been excluded as a transmitter, the men responsible for trapping the mosquitoes were instructed to concentrate on the trapping of all blood-filled female mosquitoes. During the two months a total of 984 of *C. quinquefasciatus* and 415 of *Aëdes aegypti* were caught and 84 per cent of the former and 19 per cent of the latter were blood-filled females. The results, insofar as prevention of dengue is concerned, were inconclusive and the experiment will be repeated during the coming dengue season, special attention to be given to the females of *A. aegypti*. The explanation for the higher proportionate catch of *Culex* mosquitoes is that these are less difficult to trap and most of them were caught inside mosquito nets in the early morning hours after reveille.

The total daily catch of *Culex* mosquitoes ranged from 3 to 66, while that of *Aëdes* mosquitoes varied from none to 36. Determination of species showed an overwhelming preponderance of *A. aegypti* and *C. quinquefasciatus* throughout the period. As a matter of fact, other species were observed only occasionally. For two days (August 6 and 7), a few specimens of *Mansonia* Blanchard were found in the collections, and occasionally a stray *Culex* of a species other than *C. quinquefasciatus* was noted.

Experiments with *C. quinquefasciatus* were necessary in view of the suggestive positive transmission experiments with this species reported by Ashburn and Craig (1907).

Complete and exact descriptions of *Aëdes aegypti* and of *Culex quinquefasciatus* are given in the monograph by Dyar (1922) on the mosquitoes of the United States. The mosquitoes used by us agreed with these descriptions, and the pertinent sections from that work are reprinted in the Appendix.

Breeding technic adopted.—As the series of experiments to be undertaken presupposed the availability of large numbers of mosquitoes at all times, it was necessary to develop breeding methods that would insure a consistently large output of adults. It is not difficult to breed a few mosquitoes from eggs, but when a particular series of experiments hinges on the availability of several hundred female mosquitoes during a limited period (four or five days), and when infection of the mosquitoes with a disease hinges, in turn, on the occurrence of the disease in experimental subjects, it is necessary not only to plan well ahead, but also to take every precaution so that the breeding technic in use will not fail at the crucial moment.

A review of the literature on the food requirements of the larvæ of *Aëdes aegypti* and *Culex quinquefasciatus* suggested that one or the other of the species might feed on any one of a number of kinds of food—decaying organic matter (plants or animal substances undergoing putrefaction), carbohydrates (bread crumbs, sugar, banana, etc.), bacteria, sewage, etc. Many experiments were made with foods falling into the abovementioned classes with the view to the selection of the food that would give the most consistently uniform results and the largest yield of adults.

It is to be remembered that our experiments in breeding methods were carried out in the Tropics, and it is conceivable that different methods might produce better results in temperate or colder climates.

The breeding habits of the two species under investigation differ materially, but it was hoped that one food could be found that would meet satisfactorily the food requirements of the larvæ of both species. As a matter of fact, one type of food (blood serum) was found that proved to be satisfactory.

Breeding experiments with decaying organic matter.—In making this series of experiments two methods of procedure were followed: In the first, the food was prepared in the laboratory by placing dead leaves, grass, etc., in the bottom of a jar of tap water, allowing it to stand for several days, and then introducing the lots of eggs. When the larvæ hatched out the rapidity of development was observed. The results obtained were quite unsatisfactory and inconsistent due to the unsuitable food supply. In some instances the larvæ showed a normal rate of growth for four or five days after hatching, but then died. Pupation and emergence frequently could not be forced, even though additional food was supplied.

The second method of procedure was to obtain water from pools that were known to be favorite breeding places for *Culex quinquefasciatus*, strain the water through cheesecloth to remove coarse particles, place it in breeding pans, and float the egg masses on to the surface. The larvæ hatched out, rapidly increased in size, and sometimes a considerable number pupated and emerged, but in most instances there was a cessation of growth after four or five days. If five to ten egg masses were placed in a breeding basin and the amount of water from the pool increased proportionately, the larvæ hatched and grew normally for from three to four days and then died. The food content, being dependent on a number of uncontrollable factors, apparently varied within wide limits.

The protocol of breeding experiment 147, presented in the Appendix, illustrates the sequence of events that frequently was noted.

The inconsistent results obtained with this type of food (decaying organic matter) and the low yields of adults led us to exclude this method of breeding.

Breeding experiments with dilutions of urine.—Frequently the statement appears in the literature that cesspools and collections of water containing fæces and urine constitute excellent breeding places for *Culex quinquefasciatus*. In Manila, where cesspools are much used, it frequently has been observed that they are favorite breeding places for this species.

To test out this type of food, a few experiments were made with 2 per cent solutions of urine in tap water. The following typical example of results obtained is cited:

Experiment 24: On January 25 a *Culex* egg mass containing 157 eggs (laid January 24) was placed in a breeding jar containing 2 per cent urine. Eggs hatched on the following day, and for five days the rate of growth was normal. On February 1 the hydrogen-ion concentration (p_H) value of the solution was 8.4. Beginning with February 5 the mortality rate was high, and on February 11 only 12 larvæ remained alive. On March 15 the last larva died. None of the larvæ pupated. Additions of urine during the course of the experiment did not stimulate growth.

Breeding experiments with fæces were not attempted.

The very poor results obtained in our initial experiments with this type of food led us to eliminate it from consideration.

Breeding experiments with bacteria.—In this series of experiments *Escherichia coli* (*B. coli communis*) was selected as food, with the purpose of approximating, as nearly as possible, natural conditions. The eggs of both *Culex quinquefasciatus* and *Aëdes aegypti* were used; distilled water was placed in the breeding jars and live cultures were added in some experiments and dead ones in others, and more food was added from time to time. The results obtained were quite unsatisfactory, and this type of food was eliminated from consideration.

Protocols of breeding experiments 58, 106, and 112, presented in the Appendix, indicate the details of the breeding methods adopted and exemplify the best and the poorest results obtained.

Breeding experiments with peptone.—In one experiment (No. 60), 5 per cent peptone was added to 250 cubic centimeters of distilled water and the attempt was made to breed *Culex* from an egg mass. The eggs (142) hatched, and the larvæ did well for a day or so. Thereafter, however, the solution became highly contaminated with bacterial growth, turbidity was marked, and there was a very perceptible odor suggestive of putrefactive changes. Eight days after the larvæ had emerged all were dead.

Breeding experiments with saccharose and dextrose.—Breeding experiments with these sugars gave negative results and the sequence of events noted was quite similar to that observed in the experiment with peptone, except that no putrefactive changes occurred and the contaminating organism apparently was a mold.

Breeding experiments with bread crumbs.—This type of food was used rather extensively in breeding both *Culex quinquefasciatus* and *Aëdes aegypti.* Very good results were obtained in many experiments, particularly with *A. aegypti,* but failures also were common. The principal source of trouble was molds, which are very common in the Philippines, particularly during the period of rains and high relative humidity, usually from May to October, inclusive.

If the bread was added from day to day in only sufficient amounts to meet the immediate requirements of the larvæ the output of adults frequently was satisfactory, but this was difficult to regulate. In the early stages of the experimental work crumbs from the center of the loaf of bread were used, as well as the crust, but the percentage of experiments showing contamination with molds was high.

The technic adopted and results obtained with bread crumbs are exemplified in breeding experiments 18, 62, 67, 116, 125, and 156, presented in the Appendix to this report.

Breeding experiments with blood serum.—One gains the impression from the literature that the most suitable food for *Aëdes* and *Culex* larvæ is one rich in protein; at the suggestion of Dr. Otto Schöbl, chief of the biological division, Bureau of Science, normal horse serum was selected for experimental use. The preliminary experiments gave promising results. When the details of technic had been perfected the yield of adults from a given number of both *Aëdes* and *Culex* eggs was uniformly so high that this food (blood serum) was selected for the routine breeding work.

In the early stages of experimental breeding blood serum alone was added to the stock solutions of water, but the results were not uniformly good. At times the yield of adults was large, while at other times bacterial contamination killed many, and at times all, of the larvæ or pupæ.

This complication occurred with greatest frequency when there was an excess of blood serum in the late larval stages, as the larvæ became less active and took less food preliminary to pupation.

On the assumption that it might be possible to inhibit the growth of bacteria for a week or ten days, without injury to the larvæ, many experiments were made with chemical antiseptics. In this series of experiments, sodium benzoate (0.05 to 0.1 per cent), chlorine (1 to 2 parts per million), and formalin (1: 2,500 to 1: 20,000) were used. The last, formalin, consistently gave the best results.

The routine technic finally adopted for breeding larvæ was as follows:

Aëdes larvæ.—A breeding jar (1-quart fruit jar) containing 250 cubic centimeters of tap water was placed in the egg-laying breeding cage. A few hours after eggs were laid, the jar was removed. Preferably not more than 50, and in any event not more than 75 to 100, eggs were allowed to remain in one jar.

After the hatching of the eggs, which practically always occurred on the second to the third day, from 0.5 per cent (1.25 cubic centimeters) to 1 per cent (2.5 cubic centimeters) normal horse-blood serum containing 1: 2,500 formalin was added.

The rate of growth of the larvæ was observed from day to day and, if at the end of three or four days the development began to slow down, more blood serum (0.5 to 1 per cent) was added. If at the end of another four or five days pupæ had not begun to appear, further blood serum was added, care having been taken to decrease the amounts in the late larval stages.

When this technic was followed the time interval between hatching and emergence of the first adults usually ranged from eight to twelve days and adults continued to emerge for the next five to ten days. In one experiment, in which 2 per cent blood serum was added and repeated in three days, we succeeded in breeding out a few very large adults five days after the eggs hatched. In other instances, particularly where large numbers of larvæ (100 to 150 or more) were allowed to remain in one breeding jar, the yield of adults was lower, and the imagines that finally emerged were smaller and less hardy than the average adult *Aëdes*.

If not more than 50 eggs are placed in a breeding jar and the first adults fail to appear in from eight to twelve days after hatching, the breeding technic is faulty and contamination has occurred or the food supply is inadequate.

It should not be inferred that the breeding technic adopted was uniformly successful. At times bacterial contamination, as manifested by cloudiness of the breeding solution, occurred and the yield of adults was either nil or negligible. It is our experience, however, that adherence to the method we used usually assures a yield of from 50 to 75 per cent imagines from a given number of eggs, and there is no doubt in our minds that this percentage greatly exceeds the yield under natural conditions even when the food supply is adequate and environmental conditions presumably are favorable.

Occasionally blood sera will be encountered in which the globulin content is high and the tendency for the globulin to precipitate is marked; sera showing this characteristic did not prove to be satisfactory as food. It is, of course, possible to bring the globulin back into solution by the addition of sodium chloride, but sera exhibiting this characteristic are encountered so rarely that, for practical purposes, it is well to discard them.

Culex larvæ.—Blood serum also was used in experiments on breeding the larvæ of *Culex quinquefasciatus,* and this type of food proved to be satisfactory. The steps taken in perfecting the technic were similar to those described for *Aëdes* breeding, with the following exceptions:

The biting habits of *Culex quinquefasciatus* were found to be so erratic and the death rate after egg laying so high that it was not practicable to secure supplies of eggs from females in captivity, as was done in the case of *Aëdes.* The egg masses used for breeding were collected from pools of stagnant water, which were found to be favorite places for the deposition of eggs of this species under natural conditions. Eggs were collected for breeding purposes every three to four days throughout the course of the experimental work, and this procedure assured continuous supplies of recently emerged imagines.

From four to six *Culex* egg masses were floated on the surface of the water (2,000 cubic centimeters) in a photographic developing tray. Counts of 328 egg masses made by us indicate that under natural conditions in the Philippines the *Culex* egg mass averages 188 eggs. Each tray, therefore, received approximately 1,000 eggs.

The breeding pan was then placed in the type of breeding cage shown in Plate 3, fig. 1.

When the eggs began to hatch, which practically always occurred in twenty-four hours, the food supply (blood serum) and formalin were added in the same relative proportions as for *Aëdes* breeding. The addition to the 2,000 cubic centimeters of tap water of 0.5 per cent blood serum required 10 cubic centimeters serum and formalin in proper proportions. Evaporation of water was rapid on account of the large exposed surface, and it was found to be very important to keep the water level constant by additions every two to three days.

All lots of eggs in the series had begun to hatch within twenty-four hours. Pupæ began to appear in from four to thirteen days after the hatching of the eggs, the average time being seven days. The first adults were observed in from six to seventeen days after the eggs hatched, the average being nine days. The yield of living imagines from a given number of eggs varied from 13 to 62 per cent, the average yield being 34 per cent.

The technic followed and the results obtained in the complete series of breeding experiments with blood serum as food are exemplified in protocols 30, 49, 68, 178, 182, 186, 194, 225, 230, 236, 241, and 386, presented in the Appendix.

Breeding experiments with banana.—When our earlier breeding experiments were undertaken, we had no knowledge of previous work of the same kind in which banana was used as food for the larvæ. The report of Fielding (1919) escaped our notice until early in 1925. In February, 1925, it was feasible for us to make some tentative experiments in breeding larvæ with this type of food, and the results obtained were so promising that a somewhat extensive series of experiments was undertaken. In the first series, blood serum and formalin were added to the stock breeding solution (tap water); in the second, thin slices (6.35 millimeters) of banana were added; and in the third, formalin and thin slices of banana. The eggs were collected from those deposited by stocks of females in breeding cages during a period of twenty-four hours. Eggs collected from a single breeding jar were divided into three portions and from 50 to 150 were counted and transferred to each of three breeding jars to cover each of the three experiments. The food and the formalin were not added until the eggs had hatched. The series included twenty-five experiments made with blood serum and formalin; twenty-four, with banana; and twelve, with banana and formalin.

In the twenty-five breeding experiments with blood serum as food, to which dilute solutions of formalin were added, the larvæ averaged six days to pupation and eight days to emergence of the first adults; and, of a given number of eggs, 61 per cent yielded living adults.

In the twelve experiments with banana as food, to which a weak solution of formalin was added, the larvæ averaged six days to pupation and nine days to emergence of the first adults; and, from a given number of eggs, only 55 per cent of living adults were obtained.

In the twenty-four experiments with banana alone as food, the larvæ averaged only five days to pupation and seven days to emergence of the first adults; and, from a given number of eggs, 78 per cent living adults were bred.

It was noted also that when banana alone was used as food the resulting adults were, as a rule, larger than those obtained by other breeding methods. Dilute solutions of formalin apparently inhibited growth during the larval stage. But little is known of the exact food requirements of these insects during their larval stage but, theoretically, there are reasons for assuming that a food with a fairly high protein content will best meet their needs. It is quite possible and probable that the protein content of the banana will suffice and that it contains the other food elements—carbohydrate, fat, and salt—in sufficient and sufficiently balanced proportions. So far as we have been able to determine, the vigor and stamina of adults fed on banana during the larval stage are the same as in those fed on blood serum.

The use of banana as food during the larval stage involves the application of a simple technic as compared with the use of blood serum and, in the light of our present knowledge, we should strongly advise the use of the former in breeding *Aëdes aegypti* and *Culex quinquefasciatus.*

METHODS ADOPTED FOR HANDLING ADULT MOSQUITOES

All mosquitoes used in our experiments were bred from the egg, and access to human beings for feeding purposes was completely controlled.

One reserve cage was provided for each species and as the adults emerged in the small breeding cages they were caught daily in test tubes and transferred (see Plate 5). The assistant responsible for their transfer wore a rubber glove drawn well over the cuff of the blouse to render impossible his being bitten. Males in large numbers were always present in the reserve cage, thus assuring fertilization.

Food supply.—Reserve supplies of mosquitoes, as well as several lots of infected mosquitoes, were fed on a 5 per cent aqueous solution of sugar. The food was kept in open Petri dishes, one dish to each cage, and an additional dish containing tap water was supplied. Experiments were made with banana as food, but the aqueous solution of sugar proved to be more satisfactory. It was found to be advantageous to place a folded strip of glazed paper in the dishes, on which the insects could alight when taking food or water. This reduced the mortality from drowning.

The food supply was completely changed every two days by removing the dish and replacing it with a clean Petri dish containing a fresh solution (Plate 6). Frequent changing is advisable for, should the food supply become concentrated and sticky from evaporation of the water, mosquitoes alighting on it may become entangled therein and drown; furthermore, it will tend to avoid contamination which may result from the growth of bacteria and molds.

Time interval between emergence of the mosquitoes and their use for experimental purposes.—As a rule, *Aëdes aegypti* mosquitoes were used for experimental purposes in from two to seven days after their emergence. Newly emerged *Culex quinquefasciatus* mosquitoes were held for five days and, if not used within that period, were discarded. If kept for longer periods, the death rate is rather high, after the first feeding of blood and subsequently when eggs are laid.

Technic used in feeding on human subjects.—When females of either species were required for experimental use, the proper number of clean, lightly plugged test tubes were first placed inside the reserve cage. The operator then donned a rubber glove with cuff extending well over the lower end of the sleeve of the blouse and introduced his hand and arm into the cage. Only one female was caught in each test tube. Immediately after removal of the catch the species and sex were determined and the entire lot was turned over to Mr. W. Schultze, consulting entomologist for the board, for confirmation of specific determination. As a matter of fact, no error in identification occurred throughout the course of the work.

The lot was then placed in a cage of the standard type adopted for biting experiments (see Plate 7, fig. 1), taken to the hospital, and applied to the leg of the patient. Application to the leg instead of to the arm permitted the maximum degree of freedom of movement on the part of the individual being bitten. In introducing and withdrawing the leg from the cage every precaution was taken to prevent the escape of mosquitoes. When the cage was removed, the number of mosquitoes in it was carefully checked and the cage returned to the laboratory. After reaching the laboratory lightly plugged test tubes were introduced and all females not showing complete distention of the stomach with blood were removed, counted, and killed. The blood-filled females remaining in the cage were then counted, and the total number was verified. The removal of all insects not showing complete distention of the stomach with blood assured us that all females remaining in the cage were potentially infected.

The meaning of the term "complete distention of the stomach," as used above, is the following: When a mosquito is allowed to take a full feeding of blood, the stomach becomes enormously distended and casual inspection without the use of a magnifying glass leaves one in no doubt that a full meal of blood has been taken. If, however, the insect is disturbed for one reason or another and engorgement is not complete, the stomach will show varying degrees of distention and at times it may be questionable whether or not the distention was caused by the taking of blood. To be certain, therefore, that large quantities of blood had been taken and that potential transfer of the virus had been effected, it was our custom to remove and kill all insects showing only partial distention, even though there was no question in our minds that a considerable amount of blood had actually been taken.

In the transmission experiments with *Culex*, all mosquitoes in the cage after the initial meal of blood had been taken were caught in test tubes and the blood-filled females were identified for the third time. This gave us an additional check against the possibility of any *Aëdes aegypti* mosquitoes having been introduced inadvertently. The blood-filled females, after verification of the species, were returned to the biting cage. Food and water were then introduced and the cage, properly labeled, was placed with the stocks of infected mosquitoes (see Plate 7, fig. 2).

The method of handling the lots of infected mosquitoes for the purpose of producing dengue in volunteers was exactly the same as that just described for exposure to dengue patients of newly emerged but uninfected mosquitoes for the purpose of transferring the virus to the insects, except that all the mosquitoes used were presumably infected and none were discarded after the secondary biting experiments.

Biting habits of Aëdes aegypti Linnæus.—The female of this species bites freely throughout the daylight hours in Manila and, if hungry, not infrequently it is observed biting after dusk. Though most of our biting experiments were made during the morning hours, usually between 9.30 and 11.30 a. m., we had no difficulty with them at any time during daylight hours. We did find, however, that when a biting experiment was attempted with any lot of mosquitoes that had emerged during the preceding twenty-four hours not more than 50 to 75 per cent would take blood, whereas biting experiments made with lots one or more days after emergence were entirely successful, and usually 100 per cent would take blood.

To obtain the best results in biting it is essential, therefore, that copulation take place and that the insect be allowed to take an initial meal of food (sugar or banana). The same requirement has been reported of *Anopheles* (Harold, 1923).

In one series of biting experiments to produce dengue we had planned to use the same lot of infected *Aëdes aegypti* mosquitoes every three days; but the proportion that would take blood at three-day intervals was so low—in one or two experiments nil—that this method of procedure was abandoned. The interval between blood feedings was then extended to four days and during this period water was allowed to remain in the cage, but the food supply (aqueous solution of sugar) was withdrawn. This policy was immediately abandoned as we found that, even though a full meal of blood had been taken, the death rate was rather high on the third night after the blood meal, if no food (sugar) was available. The routine method finally adopted was to allow an interval of not less than four days to intervene between biting experiments and to withdraw the food supply (but not the water) on the night of the third day, if the insects in the lot were to be used for a biting experiment on the following day.

These and other observations made by us suggest definitely that, under natural conditions, even so highly domesticated a mosquito as *Aëdes aegypti* requires, and probably exists very largely on, fruit juices.

In the early stages of the work only 10 to 15 females were allowed to bite patients with dengue for the purpose of infecting the insects; but we soon learned that, if the lots were to be held for long periods of time, it was necessary to increase the numbers materially to make up for losses due to natural death and to accidental death from crushing. Throughout the major part of the investigations 20 and preferably 25 to 30 *A. aegypti* mosquitoes were used in each lot for initial infection of the mosquito.

Biting habits of Culex quinquefasciatus Say.—The female of this species is difficult to handle, as its biting habits are very erratic. Several preliminary experiments were undertaken so that we might gain some information on this point. With the exception of one biting experiment, out of the large number made, the females refused to take blood during daylight hours, even when the cage was covered with black cloth to simulate night and the experimental subject selected for feeding purposes was known to be particularly attractive to mosquitoes.

In night biting experiments, the cage was applied at about 8 p. m. At times, a fair proportion of females had taken blood by 11 p. m., but very frequently it was necessary to leave the cage in place throughout the night and even then the biting might or might not have been satisfactory.

In six instances from 75 to 100 newly emerged *Culex quinquefasciatus* insects were applied to dengue patients with the purpose of infecting the insects. Those that took blood averaged 42 per cent, the minimum being 8 per cent, and the maximum 67.

When these lots of potentially infected *Culex quinquefasciatus* mosquitoes were again applied to experimental subjects to induce dengue, still greater difficulties were experienced. In the first place, 21 per cent of the mosquitoes had died by the time the period of development of virus in the mosquito had passed and of those remaining only 30 per cent (68 of 225) would take blood. In some instances in our initial experiments it became necessary to apply the cage on two or three successive nights.

The erratic biting habits of this species in captivity led us to adopt the general policy of using 100 females to the lot for initial biting experiments in which it was aimed to infect the mosquito.

Natural enemies of mosquitoes.—In tropical countries mosquitoes have many natural enemies and those giving us greatest concern were the small so-called jumping spiders of the family Attidæ (*Attus* sp.?) and the common red ant (*Monomorium indicum* Forel).

During one period the experiments were jeopardized because of infestation of a number of cages with spiders. During this period inexplicable shortages occurred now and then. Close observation revealed the presence of spiders, which had concealed themselves in small openings in the framework from which they emerged at infrequent intervals to obtain food. They were noted catching mosquitoes. It was possible also for the small ants to gain entrance through the large-mesh netting, and they were observed carrying dead mosquitoes away.

Incursions by ants were expected in the beginning of the work, and the legs of tables on which stocks of mosquitoes were stored were painted with a narrow band of a sticky preparation used in the manufacture of "sticky fly paper" (12 parts of castor oil and 29 parts of rosin). Notwithstanding the use of castor oil (nondrying) the preparation dried out and had to be renewed at frequent intervals (every two to three weeks). It did not constitute a barrier against the jumping spiders, as they can jump a distance of 12 to 15 centimeters. We then applied the sticky preparation to the entire length of the legs and coated the undersurface of the table tops. These spiders are ubiquitous and their habits make them difficult to control; we have observed them dropping from the ceiling of the room to the cages.

Losses of mosquitoes through the incursions of ants and spiders ceased when the measures just described were put into effect and when, in addition, all cages were re-covered with fine netting and sterilized with steam under pressure prior to use. Lack of attention to these details may entail the loss of infected mosquitoes that are difficult or impossible of replacement.

Length of life of Aëdes aegypti and Culex quinquefasciatus in captivity.—A fair proportion of laboratory-bred *Aëdes* mosquitoes lived in captivity for approximately six weeks to two months, when bred under a favorable environment. For the first five months of these experiments we had no difficulty in keeping two-thirds (66 per cent) of the various infected lots alive for thirty days, and a few individuals in each lot lived for much longer periods (sixty to seventy-five or even one hundred days). Several of our infected *Aëdes* mosquitoes remained alive for ninety-seven to ninety-nine days after infection and one lived one hundred one days; they had emerged five or six days previous to taking the infective blood. In one instance, we transmitted dengue fever with mosquitoes that had been infected seventy-five days previously.

In February and March, 1925, the early part of the dry season in Manila and a season of comparatively low temperature, the mortality rate of some lots of infected mosquitoes was high during the first ten days after their first meal of blood; it ranged from 35 to 40 per cent, and probably was attributable to comparatively low temperatures during the nights, decrease in relative humidity, and lack of stamina in the adults resulting from adverse environmental conditions during the larval stage. Subsequent to the first ten days after initial blood feeding the death rate was the same as is normally encountered among lots in captivity.

In captivity, *Culex quinquefasciatus* lives for comparatively short periods of time. In our first transmission experiments with this species we planned to begin the biting experiments on the second day after potential infection of the mosquitoes and to repeat them on the same volunteers at intervals of two or three days until at least the fifteenth day after they had bitten infected individuals. In five experiments in which this plan was followed the death rate was so high that practically all the females were dead on the twelfth day after having taken blood from a patient, and most of them died by the tenth day. Expressed in general terms, our experience with this species suggests that a considerable proportion of females, under natural conditions, take their initial meal of blood, lay their eggs, and then die, and that among those surviving subsequent to the laying of eggs the mortality rate is very high after the second and third meal of blood.

Later, when the transmission experiments with this species were repeated, we held our lots of potentially infected mosquitoes for from twelve to twenty-two days before we allowed them to bite, for the purpose of testing their ability to transmit the infection. When used for this purpose they died off rapidly when eggs subsequently were deposited.

In some of our early experiments in breeding and handling this species we attempted to carry the mosquitoes through successive generations, as can readily be done with *Aëdes aegypti* insects; but, owing to their erratic biting habits and the high death rate after blood taking, we succeeded in carrying them only to the third generation. That the death rate is high under natural conditions, when environmental conditions are adverse, is evidenced by the following observation. In attempting to start our breeding from the wild mosquito we had blood-filled females caught in test tubes a few hours after they had taken a meal of blood. Each female was then transferred to an individual egg-laying jar that contained water. In some of our early experiments we were able to obtain egg masses from every individual on the fourth or fifth day, but frequently the female would die after deposition of eggs.

During the dry season (March, 1924), when the moisture content of the air was low, we repeated this experiment, using twelve blood-filled female *Culex quinquefasciatus* mosquitoes. To our great surprise we were able to obtain only one mass of eggs from one of the females, the remaining eleven having died before they could even deposit eggs.

These and other observations suggest to us that the span of life of mosquitoes of this species in nature is short, probably not exceeding two or three weeks. The number of eggs laid by the *Culex* mosquito, compared with the *Aëdes,* is large, and probably it is this factor together with its ability to withstand low temperature (hibernation) that enables the former to maintain itself in such large numbers throughout the world.

These observations—the erratic biting habits of the *Culex* mosquito and its comparatively short span of life—suggest that it is poorly adapted to the transmission of a disease the etiological agent of which requires a somewhat prolonged developmental period in the body of the mosquito, as will later be shown to be the case in dengue.

Miscellaneous observations.—Contrary to statements that appear in the literature, it has been our experience that *Aëdes aegypti* eggs will always hatch in distilled water; but we have not, so far, seen the larvæ pupate when kept in sterilized distilled water. The following negative observations relating to the possibility that *A. aegypti* may lay eggs without having had a feeding of blood are worthy of record.

For a period of nine months, we had under daily observation thousands of male and female *Aëdes* mosquitoes in our reserve cage; the females were fertilized, none had ever taken blood, and at no time were eggs ever found in this cage. An open Petri dish containing water was always available for oviposition.

In breeding out the larvæ of both *Aëdes* and *Culex* the p_H (hydrogen-ion concentration) value of the breeding solution was apparently of no great importance, provided there was sufficient alkalinity to inhibit the development of molds.

When batches of larvæ begin to pupate and adults to emerge, the males appear first and in larger proportions. If the environmental conditions are adverse in the larval stage and only a few finally pupate and emerge, almost invariably the imagines that finally reach the free-flying stage are females. This indicates that the male larvæ are more precocious than the females but are less hardy.

The males of both species frequently, and in nature probably usually, die in from two to six days. The blood-filled female *Aëdes* will continue to live for from three to five days without food or water. The newly emerged female *A. aegypti* will seldom live for more than one day without food or water, but will live for approximately two days without food if water is available. The freshly emerged female *Culex quinquefasciatus* lives for approximately one day without food or water, but will live for two days without food if water is available.

The protocols presented in the Appendix include all lots of mosquitoes used in these investigations, exemplify the method adopted for recording observations on the various lots used experimentally, and bring out some of the points discussed in the immediately preceding pages.

PROCUREMENT OF VOLUNTEERS FOR DENGUE TRANSMISSION AND METHODS ADOPTED IN DEALING WITH THEM

In planning the transmission experiments it was impossible to escape the conclusion that clear-cut and definite results would be impossible of attainment, within a reasonable period of time, unless human subjects could be obtained. It was necessary to decide, therefore, as to whether there was sufficient justification for calling for volunteers (military personnel). It was decided that there was ample justification, and this decision was based on the following facts:

> Dengue fever is one of the four most-important causes of sickness in American troops on duty in the Philippines, and occasionally the leading cause. Any evidence that could be obtained relative to the mechanism of its transmission by mosquitoes could be applied practically in its prevention.
>
> Under existing conditions a large proportion of American troops arriving in the Philippines contract dengue fever soon after arrival and the production of dengue experimentally in such individuals, in many instances, is merely anticipating the natural course of events.
>
> The clinical course of dengue fever is short (approximately three to four days), usually the symptoms are mild, convalescence practically always is rapid, and there are no permanent after-effects.
>
> Though many thousands of cases of dengue have occurred among American troops in the Philippines during the past twenty years, no person has died of the disease.

Procurement of volunteers.—The desirability of undertaking the investigations and the justification for calling for volunteers as outlined above were set forth in a letter to the Commanding General, Philippine Department, and his approval of the investigation and assistance in obtaining the necessary volunteers requested. The entire project was approved by him and the coöperation of commanding officers was authorized and urged as is indicated in the letter constituting Exhibit A of the appendix.

During the course of the investigation sixty-four volunteers proffered their services, and they were used in transmission experiments. Their distribution by organization and station was as follows:

Post of Manila; Thirty-first Infantry	12
Fort William McKinley:	
Fifteenth Infantry	12
Sixtieth Artillery Battalion	7
Sternberg General Hospital; Medical Department Detachment	3
Camp Nichols; Air Service	11
Fort Mills; Fifty-ninth Artillery Battalion and Medical Department Detachment	19
	64

Official commendation accorded volunteers.—It was felt that the men who voluntarily subjected themselves to the bites of infected mosquitoes were deserving of the highest commendation. Not only were many of them bitten repeatedly (from two to four or more times) by various lots of potentially infectious mosquitoes, but also, when occasional negative results were obtained that, theoretically, should have been positive, they cheerfully volunteered to take subcutaneous injections of infected citrated blood for the purpose of demonstrating immunity. Furthermore, between two and three months after their recovery from dengue, practically all the volunteers in whom experimental dengue had been produced volunteered to receive subcutaneous inoculations of infected citrated blood from dengue patients for the purpose of ascertaining the duration of immunity following an attack.

A letter recommending commendation was addressed to the Department Commander (see Exhibit B, Appendix).

The names of one hundred men appear in the General Order. The additional names include the men, principally Filipino troops, who volunteered to receive inoculations of dengue-infected blood in a series of immunity experiments undertaken by the board, the results of which are presented by one of us (A. P. H.) in another section of this report.

Requirements laid down for acceptance of volunteers.—In selecting and accepting volunteers, the following general requirements were laid down:

Freedom from disease.
Short residence in the Tropics, preferably less than three months.

Previous residence in the United States in geographical areas where dengue fever had never occurred as an endemic or epidemic disease.

No record of admission to hospital for dengue fever and a negative medical history suggestive of a previous attack.

Negative history, examination for syphilis, and two negative Wassermann tests.

The volunteers accepted conformed in general to the above requirements, though occasional deviations therefrom were necessitated for one reason or another. In the late stages of the experimental work (February, 1925), when most of the troops were on field duty, it became necessary to accept a few volunteers with more than one year's residence in the Philippines, but they were drawn from Fort William McKinley and Fort Mills where dengue fever but seldom occurs in epidemic proportions.

In making investigations of the nature of those presented in this series of reports, in an area of endemicity, we consider it inadvisable to accept and use as experimental subjects any individuals except recent arrivals, if it can possibly be avoided. Notwithstanding the fact that we questioned each volunteer relative to a history of a previous attack and eliminated the possibility of prior admission to hospital for any condition suggestive of dengue, we occasionally encountered individuals in whom all mosquito-transmission experiments proved to be negative and in most of such instances it was possible further to demonstrate the existence of immunity by injections of citrated blood containing the living dengue virus. Fortunately, we had only five cases of this nature in our series, exclusive of three of our first experimental cases concerning whom we can express no definite opinion for the reason (entered into elsewhere in this report) that the virus had not existed in the mosquito long enough for it to be infective, or the controls were questionable. To convince ourselves, by control experiments, that we were dealing with immune individuals it became necessary for us to keep these five men in isolation for from five and a half to ten weeks. With one exception these five men had been in the Philippines for from four months to one and one-half years, and it is probable that they had gone through mild unrecognized attacks of dengue.

PLAN OF EXPERIMENTAL WARD, ITS ADMINISTRATION, AND THE MANAGEMENT OF EXPERIMENTAL SUBJECTS

Plan of the ward.—The screened experimental ward assigned the board by the Commanding Officer of Sternberg General Hospital, United States Army, was located on the second floor of the administration building; its capacity was fifteen patients and adequate toilet and bathing facilities were available within the ward. The general floor plan and views of the ward are shown in fig. 1 and in Plate 8, fig. 1.

Since dengue fever prevails as an endemic disease in Manila, it was necessary for us to take extraordinary precautions to

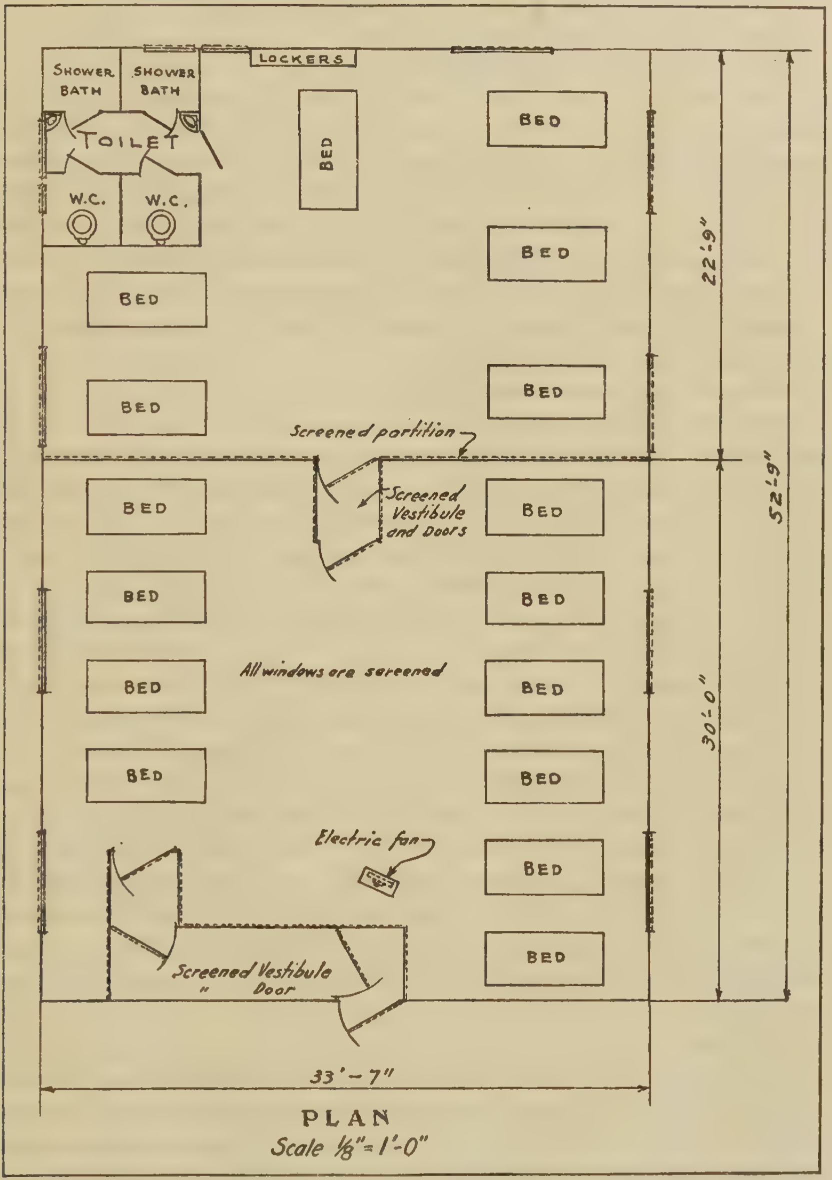

FIG. 1. Floor plan of experimental ward (Ward 16).

prevent the introduction of mosquitoes into the ward. To attain this objective, the following steps were taken:

The screens were fixed permanently to the window frames and were minutely inspected for defects, which were remedied when found. All other openings in the ceiling, walls, bath room plumbing fixtures, etc., were either sealed or screened.

Entrance was through one door, and it was made to fit closely around its edges, was screened, a vestibule was constructed inside the outer entrance door, and a closely fitting screened door formed the left wall of the vestibule as one entered. In the early stages of the work we expected to use not more than six or eight volunteers at any one time and the ward was divided into two parts by a wire-screened partition (fig. 1). Entrance to the inner ward, which housed the experimental subjects, was through a second vestibule with double-screened doors opening outward. It later became necessary to use a larger number of volunteers and double vestibules and an intervening passageway (Plate 8, fig. 2) were installed.

From the first vestibule a screened passageway, 4 feet 1 inch wide and 12 feet 7 inches long (approximately 1.25 by 4 meters), led to a second vestibule, with two additional doors, the last of which constituted the fourth barrier to the ward. The details relating to the vestibules and passageway are shown in fig. 1 and Plate 8, fig. 2. The materials used in their construction were a wooden framework covered with copper screen, 18 meshes to the inch.

All four screened doors opened outward. The interior of the vestibules and passageways was painted white, to facilitate the detection of mosquitoes that might gain entrance.

During the latter half of the period of experimental work the additional precaution was taken of installing an electric fan, the draft from which was directed toward the outer screened door.

As a preliminary measure, the entire ward was sealed and it was fumigated with sulphur, the fumes of which were allowed to remain from 4 until 9 p. m. The efficiency of the fumigation was checked by the introduction of two cages of mosquitoes, a cage being placed at each end of the ward, before ignition of the sulphur.

Administration of the ward.—The commanding officer of the hospital assigned a specially selected member of the Army Nurse Corps to administer the ward. Because of the special nature of the work and the necessity for a thorough knowledge of the unusual details required in its administration, the same nurse was retained as long as was possible. One nurse (Second Lieut. Ruby Nichols, Army Nurse Corps) was in charge from July, 1924, the date the ward was opened, until September, 1924. During the remainder of the period (September, 1924, to March, 1925), Second Lieut. Mildred P. Carter, Army Nurse Corps, administered the ward.

The commanding officer also assigned, as assistants to the nurse in charge, two ward orderlies for day duty and one ward master for night duty, all three of whom were members of the Medical Department Detachment.

One of the most important functions of the nurse in charge and her assistants was the immediate detection and destruction of any mosquitoes that might possibly gain entrance to the ward. This involved the inspection of all screens and the immediate repair of any defects found and frequent daily inspections of dark corners, the undersurfaces of beds, tables, desks, chairs, etc., and particularly of the vestibules, passageway, and bath room. A flashlight was used in making these inspections and, as different groups of volunteers were brought into the ward, their interest and active coöperation was obtained. The ward was fitted with well-trapped modern plumbing fixtures and the tops of vents were screened and kerosene oil was applied at weekly intervals to toilet flush tanks and traps in the plumbing. That the efforts directed toward the elimination of mosquitoes from the ward were highly effective is evidenced by the fact that during the entire period of the transmission experiments—July, 1924, to March, 1925, inclusive, a period of nine months—only thirteen mosquitoes were detected in the ward, vestibules, and passageway. The data pertinent to these thirteen mosquitoes are as follows:

On August 8, 1924, Miss Nichols, the nurse in charge, caught and killed a female *Aëdes aegypti* in the outer ward. On August 11, 1924, she captured and killed two additional *A. aegypti* in the outer ward, one of which took a partial feeding of blood from her, and on August 15, she killed another. Two of these mosquitoes escaped accidentally from one of the biting cages and the others doubtless gained access to the ward while additional equipment was being brought in. Two screened doors intervened between the section of the ward in which these mosquitoes were captured and the inner ward in which the volunteers were at that time in isolation. Our first positive case of transmission by *Aëdes aegypti* did not develop dengue until October 16, two months after the last of these mosquitoes was caught, and during this period careful and repeated daily search was made for mosquitoes by the nurse in charge, by the ward attendants, and by the volunteers, with negative results.

One female *Culex quinquefasciatus* was captured on September 26, and another on October 12. One of these doubtless escaped from a biting cage which checked one female short, and the other probably gained entrance through a small defect in one window screen that was repaired the same day. Neither of these mosquitoes took any blood and, furthermore, they have no bearing on the results, as this species, in our opinion, has been eliminated as a possible transmitter.

The seventh mosquito (species unidentified) was caught and killed on November 16. It entered through the bathroom window screen which, when examined the same morning, showed a defect caused by a patient leaning against it.

On March 1, 1925, four mosquitoes (unidentified) were caught and killed in the outer vestibule leading to the ward. Three doors intervened between them and the ward proper (see Plate 8, fig. 2, for location of vestibule). On the same day one female *Culex quinquefasciatus* was caught in the screened passageway leading from the outer to the inner vestibule (see Plate 8, fig. 2). On March 4, 1925, one male of the same species was caught in the ward proper.

To summarize briefly, during a period of approximately nine months only eight mosquitoes were noted in the experimental ward and five in the vestibules, and in each instance their presence could be accounted for and the portal of entry was immediately closed. Four of the eight caught in the ward were *Aëdes aegypti,* but these were detected, captured, and killed two months prior to the occurrence of the first experimental case of dengue. Only one of the entire lot (the one killed on November 15) took blood from a volunteer, and in no instance could the presence in the ward of any of these mosquitoes be connected with an experimental case.

We did not begin to obtain positive experimental cases until the latter part of the dengue season, and most of them occurred during the dry season when the mosquito population is reduced enormously and when dengue fever occurs only sporadically under natural conditions.

Management of the experimental subjects.—When a volunteer entered the experimental ward the general purposes of the investigations and the information sought in the particular experiment to be made on him were explained; thus his coöperation was elicited and his interest stimulated. He was informed that it would be necessary for him to remain in the ward until discharged by us or transferred to the regular dengue fever ward of the hospital, and the presence of ward personnel throughout the day and night assured compliance with this regulation. The volunteers were handled in the same manner as hospital patients. Though presumably in good physical condition, they were examined by the medical consultant, Major Riley. Temperature, pulse, and respiration were recorded at least four times daily throughout the entire length of their stay in the ward. Routine blood, urine, and stool examinations were made, and three Wassermann tests were done at two- or three-day intervals.

The ward was visited daily by at least one member of the board and when elevation of temperature or subjective symptoms suggestive of illness of any kind was noted Major Riley was called in.

TRANSMISSION OF DENGUE BY MOSQUITOES, II

INTRODUCTION

In the preceding section (Part I), were outlined somewhat in detail the plans formulated and put into effect preparatory to the initiation of the dengue-transmission experiments. There also were included some observations relative to the life habits of the two species of mosquitoes with which we were dealing, the technic adopted in handling them during the course of the experiments, and other pertinent information.

This section (Part II) will concern itself with the results obtained in the experiments and conclusions drawn therefrom.

Before analyzing the data at hand, it will be well to state briefly the exact information sought and the plan followed in making the experiments.

As was indicated in the introduction to Part I, our first object was to confirm the experiments of Bancroft (1906) and of Cleland and Bradley (1916) in which they succeeded in transmitting dengue fever by the use of *Aëdes aegypti* mosquitoes. The next was to determine whether or not the *Culex quinquefasciatus* mosquito is a transmitter.

Having demonstrated to our own satisfaction that *Aëdes aegypti* is a transmitter and that *Culex quinquefasciatus* is not, it was possible to initiate a series of experiments concerning the mechanism of transmission by the former. These experiments covered the following points:

Length of time required for the infected mosquito to become infective.
Period of infectivity of the dengue patient to *Aëdes aegypti.*
Length of time the infected *Aëdes aegypti* remains infective.
The possibility of hereditary transmission in the mosquito.
Miscellaneous observations.

As the method of procedure adopted in the experiments with *Culex quinquefasciatus* was based on information previously obtained concerning the mechanism of transmission by *Aëdes,* the experiments relating to *Culex* transmission are not reported in the order outlined above, but rather in logical sequence.

The transmission experiments to be reported include a total of 14 blood inoculations, 5 of which were positive; 111 biting experiments with *Aëdes aegypti,* 47 of which were positive; and 7 biting experiments with *Culex quinquefasciatus,* none of which were positive.

As the experiments were made in an area of dengue endemicity, it was necessary not only to protect the experimental subjects from the bites of wild mosquitoes while under observation, but also, before carrying out any experiments, to hold them under observation for a sufficient period of time to eliminate the possibility of any individual being in the incubation period of the disease at the time of admission to the experimental ward. In the literature it is stated that the incubation period of dengue in man may be as long as fifteen days. The incubation period of the experimentally produced cases reported in the literature ranged from two to ten days, except in one case (fifteen-day incubation) reported by Cleland and Bradley (1919–1920). Periods of incubation of less than four days occurred in individuals who were given intravenous injections of blood. The incubation period in all but one of the previously reported experimental cases produced by subcutaneous inoculations of blood and by the bites of infected mosquitoes was between four and ten days. In our early experiments, we adopted the plan of holding our experimental subjects in isolation for a period of thirteen to fifteen days, and this, plus four days' minimum incubation in man, assured us that any experiments undertaken after that time would carry us well beyond the reported maximum of fifteen days' incubation.

In work of this nature in the military service the time factor is of importance, as commanding officers of military units must train and use their personnel for military purposes. We, therefore, restudied the evidence relating to incubation periods of the disease in man and concluded that the allowance of a twelve-day incubation period would suffice to carry us well beyond any maximum period of incubation. That this estimate was accurate became evident as the number of positive experimental cases increased. The period of preliminary observation as well as the time interval between initial and secondary biting experiments, therefore, was reduced to a minimum of eight days (interval of observation, eight days, plus the minimum incubation period of four days, equals the maximum incubation period of twelve days) and that time interval was adopted as routine. That the procedure adopted was fully justified is evidenced by the fact that in 89 per cent of forty-seven cases experimentally produced, the incubation period in man was from four to seven days, inclusive. The incubation period in three instances (6 per cent) was eight days, in one instance nine days, and in one at the beginning of the tenth day.[10]

In conducting the experiments, the general policy was followed in each set of experiments of using, successively, all available methods for producing the disease and of adequately and specifically controlling all negative experiments. The following examples will suffice to explain the method adopted:

In determining the minimum time required for the infected *Aëdes* to become infective, the lots of mosquitoes were permitted to bite experimental cases on the first day of their disease. The various lots were then held for eight, nine, and ten days, and applied to volunteers. Eight or more days later, each lot used originally on a volunteer was allowed to take blood from him again, usually with positive results.

If any lot of mosquitoes, presumably infective, failed to produce dengue, other lots of *Aëdes* that had produced the disease were allowed to bite the same man, and if the results still were negative the immunity test was usually repeated by subcutaneous injections of citrated blood containing the dengue virus.

In the *Culex* experiments, lots of both *Culex* and *Aëdes* were infected from the same patient, one at night and one on the following morning, or vice versa. When the virus had had time to mature in the lot of *Culex*, the mosquitoes were applied to the experimental subject. Eight days or more afterward, the results being negative, the mosquitoes in the lot of *Aëdes* infected from the same patient during the same day were applied to the same volunteer.

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[10] The time intervals elapsing between biting experiments and the periods of preliminary observation in all our experiments will be found on page 97, Table 2, column 5, and the incubation period in each of the positive cases also is recorded, in column 16 of the same table.

In the formulation of the plan of procedure to be adhered to in conducting the initial experiments it was determined that the known facts relating to the transmission of dengue, plus the information available in the literature relative to the transmission of yellow fever by mosquitoes, would serve as a basis.

Our initial series of experiments with both *Culex* and *Aëdes* were all negative. Six volunteers were used and the mosquitoes were infected from patients in the dengue ward of the Sternberg General Hospital who had acquired the disease naturally. These patients presumably were in the second, third, and fourth days of the disease. Two of them had mild attacks, and the one in the fourth day of the disease had a well-marked terminal rash. The negative results were controlled by the injection subcutaneously of citrated blood from patients with dengue. Three of the six volunteers then came down with typical attacks of dengue, and it was from these patients that a strain of the virus was secured. We know now that the negative results obtained in our initial experiments were due to the combined influence of a number of factors, including the following:

The patients used for infecting the mosquitoes probably were in a more advanced stage of dengue than was thought to be the case at the time (soldiers with dengue seldom report themselves as sick until the second, third, or fourth day of the illness, and frequently go through a mild attack without ever reporting "sick"); the original number of mosquitoes used was too small, and by the time they had become infective, many were dead or the number remaining was inadequate for the experiment contemplated; the virus had not undergone an adequate developmental period in the mosquitoes; and, finally, three of the experimental subjects were immune.

The outstanding lesson learned from these initial experiments was the fact that it is of the greatest importance, in the conduct of experiments in insect transmission of dengue, first to produce an experimental case of the disease by the injection of blood containing the living virus, and from the experimental case so produced (concerning which all pertinent information is at hand) to infect several lots of the mosquitoes to be used in transmission work. Had this point been sufficiently appreciated by us the time devoted to transmission experiments would have been shorter and much worry would have been avoided.

When an experimental subject began to show symptoms suggestive of dengue, he was examined by a member of the board and a tentative diagnosis was made. Major Riley, the consulting internist for the board, was then called in; all diagnoses of dengue in our experimental subjects were made by him. When a positive diagnosis was made the patient was immediately transferred to the regular dengue ward of the hospital.

The clinical features of dengue are discussed in detail elsewhere in this series of reports, but it is pertinent to this particular discussion (mosquito transmission) to define what we considered the essential indications of the time of onset of the disease.

In a considerable proportion of cases of dengue the onset is sudden and the time factor can be fixed quite accurately, but in others definite subjective symptoms may precede sustained elevation of temperature by several hours, or even by a day or more. In this particular investigation we considered it desirable to use some fairly definite and easily measurable symptom; sustained elevation of temperature appearing with or following characteristic subjective symptoms was selected as the criterion.

The system adopted in assigning numerical designations to the several lots of mosquitoes and to the experimental subjects was as follows:

All lots of mosquitoes were numbered serially, 1 to 900, etc.

The volunteers used as experimental subjects also were given serial numbers, but each number was preceded by the letter A, B, or C, dependent upon the type of the experiment. A number preceded by the letter "A" indicates *Aëdes* transmission; by the letter "B," blood transmission; and by the letter "C," *Culex* transmission.

It is obvious that sometimes a single volunteer might be carried under several numbers in one or more of the three series, dependent on the nature of the experiments undertaken. Thus, Private Prock was carried as C–1, A–3, and B–2, as three transmission experiments were made on him—experimental biting by both *Culex* and *Aëdes* and final blood injection.

The results obtained in the different series of experiments are summarized in the text in tabular form and the various tables are discussed and the conclusions drawn therefrom given. Details of technic, abstracts of clinical histories, and accounts of biting experiments in each case are incorporated in the Appendix.

CONFIRMATION OF PREVIOUS REPORTS OF TRANSMISSION OF DENGUE BY AËDES AEGYPTI

Some of our early efforts to transmit by *Aëdes* mosquitoes met with no success, due almost entirely to the fact that the patients in the dengue ward of the Sternberg General Hospital used in infecting the lots of *Aëdes* were in late stages of the disease. This leads us to emphasize again the importance, in experimental work of this nature, of first producing the disease by blood inoculations and transferring the virus to the insect from this source, especially in order that there may be no doubt as to the date of onset. In our early experiments we also had the misfortune of encountering volunteers who were immune.

In later experiments, however, we confirmed the previously reported transmission of dengue fever by *Aëdes aegypti* in a total of forty-seven experimental subjects. For the sake of completeness and to form a basis for the discussion of certain phases of the mechanism of transmission the salient facts relative to all positive experimental transmissions are incorporated in Table 2.

The clinical features of these experimental cases are discussed in detail elsewhere. Suffice it to say, in connection with this particular section, that we encountered all clinical types of the disease; that in all but six, if not in all, the cases were derived from one strain of the virus; that all positive diagnoses were made by the medical consultant for the board, Maj. P. C. Riley, who was thoroughly conversant with all clinical types of dengue; and that all members of the board were in agreement on the diagnoses made.

LENGTH OF TIME REQUIRED FOR THE AËDES MOSQUITO INFECTED WITH DENGUE VIRUS TO BECOME INFECTIVE

Aëdes aegypti mosquitoes that had been infected twenty-five days previously were the transmitting agents in our first two positive experimental cases (A–12 and A–13). These cases both came down with the disease October 16, 1924. In the next four positive cases the lots of infected *Aëdes* had been infected, respectively, twenty-nine, thirty-six, fourteen, and fourteen days previously.

TABLE 2.—*Showing details of mechanism of experimental transmission of dengue.*

The experimental subject.					Mosquitoes used in experiment.		Infection of experimental mosquitoes.			Biting of volunteers by infected mosquitoes.			Results.			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
No.	Experimental case No.	Volunteer.	Admitted to experimental ward—	Days in isolation or since bitten by mosquitoes.	Serial No. of lot.	Number in lot.	Name and experimental case No. of volunteer used to infect mosquitoes.	Day of dengue in patient.	Mosquitoes were infected—	Volunteer was bitten—	Number of females that took blood.	Number of days virus had remained in mosquitoes.	Positive (+) or negative (—).	Initial symptoms appeared—	Incubation period in days.	No.
1	A-12	Yarch	Sept. 12, 1924	13	551	10	Richards B-3	1.0	Sept. 17, 1924	Oct. 12, 1924	10	25	+	Oct. 16, 1924	4.25	1
2	A-13	Crone	do	13	552	20	Reed B-1	0.75	do	do	20	25	+	do	4.5	2
3	A-18	Ables	Sept. 19, 1924	13	551 552	8	Richards B-3 Reed B-1	1.00 0.75	do	Oct. 16, 1924	4	29	+	Oct 21, 1924	5.5	3
4	A-20	Alexander	do	18	551 552	2	Richards B-3 Reed B-1	1.00 0.75	do	Oct. 23, 1924	2	36	+	Oct. 29, 1924	5.75	4
5	A-26	Carter	Oct. 20, 1924	11	663	10	Siler	0.50	Oct. 17, 1924	Oct. 31, 1924	8	14	+	Nov. 5, 1924	5.50	5
6	A-27	Sparks	do	11	664	10	Yarch A-12	1.00	do	do	7	14	+	Nov. 7, 1924	7.25	6
7	A-28	Kibbe	do	11	665	6	Crone A-13	0.75	do	do	6	14	+	do	7.50	7
8	A-33	Swaen	do	15	663	8	Siler	0.50	do	Nov. 4, 1924	7	18	+	Nov. 10, 1924	5.75	8
9	A-34	Cain	do	15	664	6	Yarch A-12	1.00	do	do	5	18	+	Nov. 8, 1924	4.50	9
10	A-35	Caron	do	15	665	6	Crone A-13	0.75	do	do	4	18	+	Nov. 9, 1924	4.75	10
11	A-40	Watkins	do	18	687	6	Ables A-18	0.75	Oct. 22, 1924	Nov. 7, 1924	6	16	+	Nov. 16, 1924	9.25	11
12	A-46	Eckenroad	do	8	694	27	Alexander A-20	0.25	Oct. 29, 1924	Nov. 15, 1924	25	17	+	Nov. 19, 1924	4.25	12
13	A-48	Byam	Nov. 13, 1924	16	724	24	Watkins A-40	0.75	Nov. 17, 1924	Nov. 29, 1924	16	12	+	Dec. 7, 1924	8.25	13
14	A-50	Riggs	do	17	725	44	Eckenroad A-46	0.25	Nov. 19, 1924	Nov. 30, 1924	34	11	+	do	6.75	14

TABLE 2.—*Showing details of mechanism of experimental transmission of dengue*—Continued.

The experimental subject.					Mosquitoes used in experiment.		Infection of experimental mosquitoes.			Biting of volunteers by infected mosquitoes.			Results.			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
No.	Experimental case No.	Volunteer.	Admitted to experimental ward—	Days in isolation or since bitten by mosquitoes.	Serial No. of lot.	Number in lot.	Name and experimental case No. of volunteer used to infect mosquitoes.	Day of dengue in patient.	Mosquitoes were infected—	Volunteer was bitten—	Number of females that took blood.	Number of days virus had remained in mosquitoes.	Positive (+) or negative (—)	Initial symptoms appeared—	Incubation period in days.	No.
15	A-52	Dembowski	Oct. 20, 1924	7	714	24	Carter A-26	0.75	Nov. 6, 1924	Nov. 24, 1924	18	18	+	Dec. 1, 1924	7.25	15
16	A-53	Squires	Nov. 11, 1924	10	694	15	Alexander A-20	0.25	Oct. 29, 1924	Nov. 30, 1924	13	32	+	Dec. 5, 1924	4.75	16
17	A-54	White	do	10	717	31	Kibbe A-28	0.50	Nov. 8, 1924	Dec. 6, 1924	30	28	+	Dec. 14, 1924	8.50	17
18	A-55	Currier	Nov. 10, 1924	10	718	33	Sparks A-27	0.75	do	do	30	28	+	Dec. 11, 1924	5.25	18
19	A-57	Working	Nov. 13, 1924	10	726	36	Eckenroad A-46	0.75	Nov. 20, 1924	Dec. 10, 1924	25	20	+	Dec. 14, 1924	4.75	19
20	A-60	St. Hill	Dec. 9, 1924	13	760	43	Dembowski A-52	1.25	Dec. 2, 1924	Dec. 22, 1924	36	20	+	Jan. 1, 1925	10.00	20
21	A-61	Calog	Dec. 8, 1924	8	757	15	do	0.25	Dec. 1, 1924	Dec. 28, 1924	14	27	+	Jan. 5, 1925	8.25	21
22	A-62	Maloy	do	8	757	14	do	0.25	do	do	12	27	+	Jan. 4, 1925	6.75	22
23	A-65	Hughes	Dec. 19, 1924	10	769	17	Riggs A-50	2.25	Dec. 9, 1924	Dec. 29, 1924	13	20	+	Jan. 6, 1925	7.75	23
24	A-69	Wach	do	14	769	16	do	2.25	do	Jan. 2, 1925	9	24	+	do	4.25	24
25	A-71	Nelson	do	14	778	20	Working A-57	2.50	Dec. 17, 1924	do	16	16	+	Jan. 8, 1925	6.25	25
26	A-72	Driscoll	do	11	717	16	Kibbe A-28	0.50	Nov. 8, 1924	Jan. 9, 1925	12	62	+	Jan. 14, 1925	5.25	26
27	A-73	Stokes	do	11	763	23	Squires A-53	0.25	Dec. 5, 1924	do	19	35	+	Jan. 13, 1925	4.25	27
28	A-74	Tanis	do	11	768	4	Byam A-48	1.00	Dec. 8, 1924	do	3	32	+	Jan. 14, 1925	5.25	28
29	A-75	Shade	do	11	772	11	Currier A-55	0.75	Dec. 12, 1924	do	6	28	+	do	5.50	29
30	A-77	Peterson	do	11	717	11	Kibbe A-28	0.50	Nov. 8, 1924	Jan. 13, 1925	10	66	+	Jan. 18, 1925	5.50	30
31	A-86	Foos	do	9	717	7	do	0.50	do	Jan. 22, 1925	6	75	+	Jan. 27, 1925	5.50	31

32	A-87	Tate	Jan. 13, 1925	8	763	16	Squires A-53	0.25	Dec. 5, 1924	Jan. 31, 1925	13	57	+	Feb. 6, 1925	5.75	32
							Peterson A-77	0.75	Jan. 19, 1925			12				
					769	9	Riggs A-50	2.25	Dec. 9, 1924		9	53				
							Peterson A-77	0.75	Jan. 19, 1925			12				
33	A-88	Boran	do	11	806	17	Tanis A-74	1.00	Jan. 15, 1925	Feb. 3, 1925	13	19	+	Feb. 9, 1925	5.75	33
34	A-89	Clark, A. L.	do	10	807	16	Shade A-75	0.75	do	do	13	19	+	do	6.50	34
35	A-90	Hockett	do	9	808	15	Driscoll A-72	1.00	do	do	10	19	+	Feb. 8, 1925	5.50	35
36	A-91	Byrd	Jan. 20, 1925	10	800	7	Hughes A- 5	1.25	Jan. 7, 1925	Feb. 7, 1925	7	31	+	Feb. 12, 1925	5.25	36
37	A-92	Barnes	do	10	801	9	Wach A-69	0.75	do	do	7	31	+	do	5.25	37
38	A-94	Brock	do	10	801	9	do	0.75	do	do	4	31	+	Feb. 14, 1925	7.50	38
39	A-96	McAllister	Feb. 5, 1925	9	811	24	Foos A-86	[a]0.50	Jan. 27, 1925	Feb. 14, 1925	23	18	+	Feb. 19, 1925	5.50	39
40	A-101	Duncan	do	14	818	9	Tate A-87	[a]0.75	Feb. 5, 1925	Feb. 19, 1925	9	14	+	Feb. 26, 1925	7.50	40
41	A-102	Clark, Wm.	Feb. 14, 1925	8	822	12	Hockett A-90	[a]1.50	Feb. 7, 1925	Feb. 22, 1925	11	15	+	Feb. 28, 1925	6.25	41
42	A-110	Jensen	Feb. 5, 1925	8	819	5	Tate A-87	0.25	Feb. 6, 1925	Feb. 24, 1925	4	18	+	Mar. 3, 1925	6.75	42
43	A-111	Deane	Feb. 14, 1925	8	828	2	Hockett A-90	0.50	Feb. 9, 1925	Mar. 2, 1925	2	21	+	Mar. 7, 1925	5.00	43
44	A-112	Essary	do	8	827	10	Clark, A. L. A-89	[a]0.25	do	do	10	21	+	Mar. 8, 1925	6.50	44
45	A-116	Hammitt	do	12	827	9	do	[a]0.25	do	Mar. 6, 1925	5	25	+	Mar. 11, 1925	4.75	45
46	A-117	Hawkins	do	8	893	13	Capello A-100	1.00	Feb. 26, 1925	Mar. 10, 1925	13	12	+	Mar. 15, 1925	4.75	46
					900	4	Hughes A-65	[a]1.25	Jan. 7, 1925		3	62				
47	A-119	Shaver	do	8	900	24	Duncan A-101	1.00	Feb. 27, 1925	do	23	11	+	Mar. 14, 1925	4.50	47
					801	8	Wach A-39	0.75	Jan. 7, 1925		5	62				

[a] Days prior to onset of dengue in patient.

The next step in our experiments, therefore, was to determine the minimum incubation period of the virus in the infected mosquito. The method of procedure adopted in making and properly controlling this series of experiments was as follows:

Several lots of *Aëdes* mosquitoes were allowed to bite experimentally produced cases of dengue during the first day of the disease. These lots were applied to volunteers seven, eight, nine, ten, eleven, and twelve days after the infection of the mosquitoes. When the experiment yielded negative results the experimental subject was again exposed to the lot used in the original attempt to infect. With two exceptions, the time intervening between the first and the second experiments was not less than eight days. The results obtained are incorporated in Table 3.

In making these experiments eight volunteers were used and in each set of experiments the lots of mosquitoes were infected from patients with dengue on the day of onset of the disease.

All six experiments made with lots of mosquitoes in which the virus had been allowed to develop for from seven to ten days, inclusive, gave negative results. The negative results in these cases were controlled positively by having the experimental subjects bitten by the same lots of *Aëdes* but in which the virus had now remained for considerably longer periods of time (seventeen to twenty days). Two experiments were made with lots of mosquitoes infected eleven days previously and in one instance the result was negative with a positive control later, and in the other dengue was successfully transmitted on initial biting by infected *Aëdes*.

Conclusion.—On the basis of the evidence presented above, we feel that we can state with confidence that the dengue virus must remain in the female *Aëdes* for a period of more than ten days before the insect becomes capable of transmitting it to human beings. The evidence indicates that even on the eleventh day after infection of the *Aëdes*, the virus may not have reached such a state that the bite of the mosquito is certainly infective.

PERIOD OF INFECTIVITY OF THE DENGUE PATIENT TO AËDES AEGYPTI

Having established the fact that infected *Aëdes* are not capable of transmitting the virus of dengue until approximately the eleventh day after their infection, it was felt that the next problem requiring solution was related to the stages of the disease during which the individual with dengue is capable of

TABLE 3.—*Experimental data indicating length of time required for Aëdes mosquitoes infected with the dengue virus to become infective.*

Experimental subject.				Mosquitoes used in experiment.		Infection of experimental mosquitoes.			Biting of volunteers by infected mosquitoes.			Results.		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
													Positive cases.	
Experimental case No.	Volunteer.	Admitted to experimental ward—	Days in isolation or since bitten by mosquitoes.	Serial No. of lot.	Number in lot.	Name and experimental case No. of volunteer used to infect mosquitoes.	Day of dengue in patient.	Mosquitoes were infected—	Volunteer was bitten—	Number of females that took blood.	Number of days virus had remained in mosquitoes.	Positive (+) or negative (—).	Initial symptoms appeared—	Incubation period in days.
A-41	Eckenroad	Oct. 20, 1924	18	694	41	Alexander A-20	1	Oct. 29, 1924	Nov. 7, 1924	30	9	—		
A-46	do	do	8	694	27	do	1	do	Nov. 15, 1924	25	17	+	Nov. 19, 1924	4.25
A-42	Dembowski	do	9	714	34	Carter A-26	1	Nov. 6, 1924	Nov. 13, 1924	31	7	—		
A-47	do	do	4	714	30	do	1	do	Nov. 17, 1924	24	11	—		
A-52	do	do	7	714	24	do	1	do	Nov. 24, 1924	18	18	+	Dec 1, 1924	7.25
A-48	Byam	Nov. 13, 1924	16	724	24	Watkins A-40	1	Nov. 17, 1924	Nov 29, 1924	16	12	+	Dec. 7, 1924	8.25
A-50	Riggs	do	17	725	44	Eckenroad A-46	1	Nov. 19, 1924	Nov. 30, 1924	34	11	+	do	6.75
A-51	Working	do	17	726	36	do	1	Nov. 20, 1924	do	28	10	—		
A-57	do	do	10	726	36	do	1	do	Dec. 10, 1924	25	20	+	Dec. 14, 1924	4.75
A-78	Boran	Jan. 13, 1925	10	806	18	Tanis A-74	1	Jan. 15, 1925	Jan. 23, 1925	14	8	—		
A-88	do	do	11	806	17	do	1	do	Feb. 3, 1925	13	19	+	Feb. 9, 1925	5.75
A-79	Clark, A. L.	do	11	807	17	Shade A-75	1	do	Jan. 24, 1925	16	9	—		
A-89	do	do	10	807	16	do	1	do	Feb. 3, 1925	13	19	+	Feb. 9, 1925	6.5
A-80	Hockett	do	12	808	17	Driscoll A-72	1	do	Jan. 25, 1925	15	10	—		
A-90	do	do	9	808	15	do	1	do	Feb. 3, 1925	10	19	+	Feb. 8, 1925	5.5

infecting mosquitoes. The method pursued in determining this point was as follows:

Experimental subjects with dengue fever were bitten by several lots of *Aëdes aegypti* on the day of onset and each day thereafter for three to four days (first to fourth or fifth day of dengue). The lots so infected were then held for sixteen to twenty-six days. Subsequently the lots potentially infected on the second, third, fourth, and fifth days of dengue were applied to experimental subjects. A period of at least eight days was then allowed to elapse, after which time, if dengue had not developed, the negative results were controlled by reëxposure of the subject to lots of *Aëdes* infected on the first day of the disease, preferably from the same dengue case. The results covering this phase of the investigation are summarized in Table 4.

Experiments were made on fourteen individuals in this series. The onset of symptoms was sudden in all except two (Working and Wach) of the experimental subjects used for infecting the lots of *Aëdes*.

Our four attempts to produce dengue in volunteers by exposing them to the lots of mosquitoes that had bitten patients on the fourth and fifth days of an attack (two experiments in each instance) were negative. Two of the patients (Hughes and Wach), used to determine their infectiveness for mosquitoes on the fourth and fifth days of the disease, still had slight elevation of temperature on the day the *Aëdes* were allowed to bite; but in the third case (Dembowski) the temperature was normal throughout the day.

The four experiments were controlled by exposing the same individuals (Calog, Byrd, Barnes, and Brock) to the lots of *Aëdes* infected from the same individuals, but during the first day of the attack; this time all came down with dengue.

Nine attempts were made to transmit with the lots of *Aëdes* that had bitten patients on the third day of dengue. Six gave negative results (Maloy, Stokes, Tanis, Shade, Foos, and Peterson), and the remaining three (Hughes, Wach, and Nelson) were positive. In five of the six negative experiments the lots of *Aëdes* took blood from patients who had definite elevation of temperature on that day. In the three positive experiments, there was elevation of temperature on the third day also in the patients used to infect the mosquitoes.

All negative results, with two exceptions (Foos and Peterson), were positively controlled by a rebiting by *Aëdes* infected from the same patients, but on the first day of their dengue. The two exceptions, which also were positive controls, were made for another purpose; namely, to demonstrate the length of time the infected *Aëdes* continue to be infective.

Having determined that individuals with dengue are infective for *Aëdes* during the first three days of the disease it was of considerable practical importance, from the viewpoint of prevention, to determine whether an individual on the verge of an attack is capable of infecting mosquitoes immediately prior to the actual onset of symptoms. Information was sought on this point in the following manner:

The volunteers were first bitten by infected mosquitoes. Then, beginning on the third day following this initial infective biting, freshly bred lots of normal *Aëdes* were allowed to bite them on each day of their incubation period. Such daily biting, using new and uninfected mosquitoes each day, was continued until the volunteer showed the first sustained fever indicative of the onset of dengue.

The lots thus potentially infected in the late incubationary stages were held in order to permit development of the virus and then, to test their infectivity, they were applied to new volunteers. The negative results were controlled subsequently by exposure of the experimental subjects to *Aëdes* infected from the same patients, but on the first day of the disease. The results obtained in this series of experiments are summarized in Table 5.

Seven volunteers were used in this series of experiments and the lots of *Aëdes* used for transmission were infected from four experimental subjects in the late prodromal stages of dengue. In all except one (Hockett) of the four patients used for the initial infection of the *Aëdes,* the onset of symptoms—a combination of subjective symptoms and elevation of temperature—was sudden, though definite subjective symptoms (headache, lassitude) appeared from six to ten hours prior to elevation of temperature. The remaining patient (Hockett) complained of headache for three days before definite onset of dengue. He also complained of feeling ill on the morning of the day of onset, but his case was not definitely diagnosed as dengue until the evening of the same day.

It may be said, therefore, that the information recorded in columns 8 and 9 of Table 2 may be relied on, except in the case of Private Hockett, who had indefinite prodromal symptoms of dengue for three days prior to definite onset.

In four instances (Jensen, Deane, Essary, and Hammitt), we failed to produce dengue by exposure to *Aëdes* potentially in-

TABLE 4.—*Period of infectivity of dengue patients to Aëdes aegypti subsequent to onset of symptoms.*

Experimental subject.				Mosquitoes used in experiment.		Infection of experimental mosquitoes.		
1	2	3	4	5	6	7	8	9
							Day of dengue in patient.	
Experimental case No.	Volunteer.	Admitted to experimental ward—	Days in isolation or since bitten by mosquitoes.	Serial No. of lot.	Number in lot.	Name and experimental case No. of volunteer used to infect mosquitoes.	Whole numbers.	Whole numbers and fractions thereof.
A-58	Calog	Dec. 8, 1924	12	762	36	Dembowski A-52	4	3.25
A-61	do	do	8	757	15	do	1	0.25
A-59	Maloy	do	12	761	40	do	3	2.25
A-62	do	do	8	757	14	do	1	0.25
A-60	St. Hill	Dec. 9, 1924	13	760	43	do	2	1.25
A-64	Stokes	Dec. 19, 1924	10	765	6	Squires A-53	3	2.25
A-73	do	do	11	763	23	do	1	0.25
A-65	Hughes	do	10	769	17	Riggs A-50	3	2.25
A-66	Tanis	do	10	771	17	Byam A-48	3	2.75
A-74	do	do	11	768	4	do	1	1.0
A-67	Shade	do	10	774	15	Currier A-55	3	2.75
A-75	do	do	11	772	6	do	1	0.75
A-68	Foos	do	14	765	6	Squires A-53	3	2.25
A-76	do	do	11	714	3	Carter A-26	1	0.75
A-86	do	do	9	717	7	Kibbe A-28	1	0.5
A-69	Wach	do	14	769	16	Riggs A-50	3	2.25
A-70	Peterson	do	14	771	17	Byam A-48	3	2.75
A-77	do	do	11	717	11	Kibbe A-28	1	0.5
A-71	Nelson	do	14	778	20	Working A-57	3	2.5

A-82	Byrd	Jan. 20, 1925	8	803	14	Hughes A-65	5	4.25
A-91	do	do	10	800	7	do	2	1.25
A-83	Barnes	do	8	805	11	Wach A-69	5	4.75
A-92	do	do	10	801	9	do	1	0.75
A-85	Brock	do	8	804	7	do	4	3.75
A-94	do	do	10	801	9	do	1	0.75

Experimental case No.	Infection of experimental mosquitoes.		Biting of volunteers by infected mosquitoes.			Results.			
	10	11	12	13	14	15	16	17	18
							Positive cases.		
								Incubation period in days.	
	Temperature (°F.) range on day mosquitoes were infected.	Mosquitoes were infected—	Volunteer was bitten—	Number of females that took blood.	Number of days virus had remained in mosquitoes.	Positive (+) or negative (—).	Initial symptoms appeared—	Whole numbers.	Whole numbers and fractions thereof.
A-58	98.6 all day	Dec. 4, 1924	Dec. 20, 1924	28	16	—			
A-61	99-101	Dec. 1, 1924	Dec. 28, 1924	14	28	+	Jan. 5, 1925	8	8.50
A-59	99-99	Dec. 3, 1924	Dec. 20, 1924	29	17	—			
A-62	99-101	Dec. 1, 1924	Dec. 28, 1924	12	28	+	Jan. 4, 1925	6	6.75
A-60	99-100	Dec. 2, 1924	Dec. 22, 1924	36	20	+	Jan. 1, 1925	10	10.0
A-64	99-100	Dec. 7, 1924	Dec. 29, 1924	6	22	—			
A-73	100-102	Dec. 5, 1924	Jan. 9, 1925	19	35	+	Jan. 13, 1925	5	4.25
A-65	99-100	Dec. 9, 1924	Dec. 29, 1924	13	20	+	Jan. 6, 1925	7	7.75
A-66	98.6-99.4	Dec. 10, 1924	do	15	19	—			
A-74	99-101	Dec. 8, 1924	Jan. 9, 1925	3	32	+	Jan. 14, 1925	5	5.25
A-67	98.6-99.8	Dec. 14, 1924	Dec. 29, 1924	10	15	—			
A-75	98.6-100.6	Dec. 12, 1924	Jan. 9, 1925	6	28	+	Jan. 14, 1925	5	5.50
A-68	99-100	Dec. 7, 1924	Jan. 2, 1925	2	26	—			
A-76	98.6-102	Nov. 6, 1924	Jan. 13, 1925	2	68	—			

TABLE 4.—*Period of infectivity of dengue patients to Aëdes aegypti subsequent to onset of symptoms*—Continued.

Experimental case No.	Infection of experimental mosquitoes		Biting of volunteers by infected mosquitoes.			Results.			
	10	11	12	13	14	15	16	17	18
							Positive cases.		
	Temperature (°F.) range on day mosquitoes were infected.	Mosquitoes were infected—	Volunteer was bitten—	Number of females that took blood.	Number of days virus had remained in mosquitoes.	Positive (+) or negative (—).	Initial symptoms appeared—	Incubation period in days. Whole numbers.	Incubation period in days. Whole numbers and fractions thereof.
A-86	97.4-100	Nov. 8, 1924	Jan. 22, 1925	6	75	+	Jan. 27, 1925	5	5.50
A-69	99-100	Dec. 9, 1924	Jan. 2, 1925	9	24	+	Jan. 6, 1925	4	4.75
A-70	98.6-99.4	Dec. 10, 1924	do	8	23	—			
A-77	97.4-100	Nov. 8, 1924	Jan. 13, 1925	10	66	+	Jan. 18, 1925	5	5.5
A-71	98.8-99.6	Dec. 17, 1924	Jan. 2, 1925	16	16	+	Jan. 8, 1925	6	6.25
A-82	97-99.4	Jan. 10, 1925	Jan. 28, 1925	13	18	—			
A-91	100.2-102	Jan. 7, 1925	Feb. 7, 1925	7	31	+	Feb. 12, 1925	5	5.25
A-83	98.4-99.2	Jan. 11, 1925	Jan. 28, 1925	10	17	—			
A-92	99.6-102.6	Jan. 7, 1925	Feb. 7, 1925	7	31	+	Feb. 12, 1925	5	5.25
A-85	99-99.2	Jan. 10, 1925	Jan. 28, 1925	5	18	—			
A-94	99.6-102.6	Jan. 7, 1925	Feb. 7, 1925	31	7	+	Feb. 14, 1925	7	7.50

TABLE 5.—*Period of infectivity of dengue patients to Aëdes aegypti prior to definite onset of symptoms.*

Experimental subject.				Mosquitoes used in experiment.		Infection of experimental mosquitoes.				Biting of volunteers by infected mosquitoes.			Results.		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
														Positive cases.	
Experimental case No.	Volunteer.	Admitted to experimental ward—	Days in isolation or since bitten by infected mosquitoes.	Serial No. of lot.	Number in lot.	Name and experimental case No. of volunteer used to infect mosquitoes.	Number of days *prior* to onset of dengue in patient.	Number of days *after* onset of dengue in patient.	Mosquitoes were infected—	Volunteer was bitten—	Number of females that took blood.	Number of days virus had remained in mosquitoes.	Positive (+) or negative (—).	Initial symptoms appeared—	Incubation period in days.
A-96	McAllister	Feb. 5, 1925	9	811	24	Foos A-86	0.5		Jan. 27, 1925	Feb. 14, 1925	23	18	+	Feb. 19, 1925	5.5
A-98	Jensen	do	11	817	13	Tate A-87	1.75		Feb. 4, 1925	Feb. 16, 1925	12	12	—		
A-110	do	do	8	819	5	do		0.25	Feb. 6, 1925	Feb. 24, 1925	4	18	+	Mar. 3, 1925	6.75
A-101	Duncan	do	14	818	9	do	0.75		Feb. 5, 1925	Feb. 19, 1925	9	14	+	Feb. 26, 1925	7.5
A-102	Clark, Wm.	Feb. 14, 1925	8	822	12	Hockett A-90	1.50		Feb. 7, 1925	Feb. 22, 1925	11	15	+	Feb. 28, 1925	6.25
A-103	Deane	do	8	826	7	do	0.50		Feb. 8, 1925	Feb. 22, 1925	7	14	—		
A-111	do	do	8	828	2	do		0.50	Feb. 9, 1925	Mar. 2, 1925	2	21	+	Mar. 7, 1925	5.0
A-104	Essary	do	8	823	10	Clark, A. L. A-89	2.25		Feb. 7, 1925	Feb. 22, 1925	10	15	—		
A-112	do	do	8	827	10	do	0.25		Feb. 9, 1925	Mar. 2, 1925	10	21	+	Mar. 8, 1925	6.5
A-105	Hammitt	do	8	825	5	do	1.25		Feb. 8, 1925	Feb. 22, 1925	5	14	—		
A-116	do	do	12	827	9	do	0.25		Feb. 9, 1925	Mar. 6, 1925	5	25	+	Mar. 11, 1925	4.75

fected from patients 1.25 to 2.25 days prior to the definite onset of dengue. In one instance (Clark, Wm. G.), an attack of dengue occurred following exposure to *Aëdes* infected from a patient 2.25 days prior to the appearance of definite symptoms of dengue; but, as was pointed out above, the patient (Hockett) used for initial infection of this lot of *Aëdes* manifested indefinite prodromal symptoms of dengue for three days before actual onset.

In two instances (McAllister and Duncan) we produced dengue by exposure to the lots of *Aëdes* infected from patients twelve to eighteen hours prior to definite onset of dengue. The four subjects (Jensen, Deane, Essary, and Hammitt) who did not contract dengue as a result of the initial experiments, were rebitten by lots of *Aëdes* infected from the same sources but in later stages (twelve hours prior to onset to six hours after onset), and all came down with typical attacks of dengue.

Conclusions.—In our opinion the evidence obtained from this series of experiments warrants the following statement:

Individuals with dengue fever are infective to the *Aëdes* mosquito during the first three days of the disease, but after the second day the mosquito fails, not infrequently, to pick up the virus.

Furthermore, individuals in the late prodromal stages of dengue—from six to eighteen hours prior to the onset—sometimes are infective to *Aëdes aegypti.*

LENGTH OF TIME THE INFECTED AËDES AEGYPTI REMAINS INFECTIVE

The information concerning the duration of infectivity in the infected *Aëdes aegypti* is included in Table 5, column 13, and is summarized in Table 6. Only forty-three positive cases are included, as three of our experimental cases resulted from exposure to two or more lots of *Aëdes.*

TABLE 6.—*Summary of data shown in Table 5.*

Lots.	Days after their infection that *Aëdes aegypti* produced dengue.	Lots.	Days after their infection that *Aëdes aegypti* produced dengue.
1	11	2	27
1	12	3	28
4	14	1	29
1	15	3	31
2	16	2	32
1	17	1	35
6	18	1	36
3	19	1	62
3	20	1	66
2	21	1	75
1	24		
2	25	43	

Positive results were not obtained until the eleventh day after infection, and from that time onward we succeeded in transmitting the virus by mosquitoes as long as seventy-five days after their infection, positive results having been obtained sixty-two, sixty-six, and seventy-five days subsequent to infection.

Conclusion.—We believe the evidence obtained justifies the statement that after the female of *Aëdes aegypti* has become infective she is capable of transmitting the virus of dengue throughout the remainder of her natural life.

ELIMINATION OF CULEX QUINQUEFASCIATUS (C. FATIGANS) AS A TRANSMITTER OF DENGUE

In making this series of experiments the plan followed was to permit lots of both *Culex quinquefasciatus* and *Aëdes aegypti* to take a feeding of blood from the same experimental case on the same day at night, and another feeding during the day. These potentially infected lots were then held for at least twelve days, the period that would be required for the mosquitoes to become infective. The experimental subjects were then bitten by the *Culex* mosquitoes and observed for the appearance of symptoms of dengue. When a period of time exceeding the incubation of the disease in man had elapsed, the negative *Culex* experiments were controlled by allowing the volunteers to be bitten by the *A. aegypti* infected from the same sources on the same day. The results obtained are summarized in Table 7.

Briefly, this table shows that in seven instances we failed to produce dengue fever in volunteers who were bitten by adequate numbers of potentially infected *Culex quinquefasciatus*. When the experiment was controlled by exposing the same volunteers to *Aëdes aegypti,* infected from the same patients on the same day, we succeeded in every instance in producing dengue. In only one of the seven experiments could the results be questioned—on the basis of insufficient inoculum—namely, in the case of the *Culex* experiment with Ables C–8 who was bitten by only two *Culex;* but, on the other hand, dengue was transmitted to another volunteer in the same series—Alexander A–20—by the bites of two *A. aegypti.*

Conclusion.—We believe the conclusion is justified that *Culex quinquefasciatus* does not transmit dengue fever.

POSSIBILITY OF THE HEREDITARY TRANSMISSION OF THE VIRUS OF DENGUE IN THE MOSQUITO

In view of the statements made by Marchoux and Simond relative to the transmission of the virus of yellow fever through

TABLE 7.—*Experimental data demonstrating that Culex quinquefasciatus is not a transmitter of dengue.*

Experimental subject.				Mosquitoes used in experiment.			Infection of experimental mosquitoes.			Biting of volunteers by infected mosquitoes.			Results.		
														Positive cases.	
Experimental case No.	Volunteer.	Admitted to experimental ward—	Days in isolation or since bitten by mosquitoes.	Serial No. of lot.	Species.	Number in lot.	Name and experimental case No. of volunteer used to infect mosquitoes.	Day of dengue in patient.	Mosquitoes were infected—	Volunteer bitten—	Number of females that took blood.	Number of days virus had remained in mosquitoes.	Positive (+) or negative (—).	Initial symptoms appeared—	Incubation period in days.
C-6	Yarch	Sept. 12, 1924	17	559	*C. quinquefasciatus*	5	Richards B-3	1	Sept. 17, 1924	Sept. 29, 1924	14	12	—		
				557	do	10	Prock B-2	1	do	do					
A-12	do	do	13	551	*A. aegypti*	10	Richards B-3	1	do	Oct. 12, 1924	10	25	+	Oct. 16, 1924	4.5
C-7	Crone	do	17	558	*C. quinquefasciatus*	13	Reed B-1	1	do	Sept. 29, 1924	7	12	—		
A-13	do	do	13	552	*A. aegypti*	20	do	1	do	Oct. 12, 1924	20	25	+	Oct. 16, 1924	4.5
C-8	Ables	Sept. 19, 1924	13	559	*C. quinquefasciatus*	5	Richards B-3	1	do	Oct. 2, 1924	1	15	—		
				557	do	10	Prock B-2	1	do	Oct. 3, 1924	1	16			
A-18	do	do	13	551	*A. aegypti*	8	Richards B-3	1	do	Oct. 16, 1924	4	29	+	Oct. 21, 1924	5.5
				552	do		Reed B-1	1	do	Oct. 2, 1924	3	15	—		
C-9	Alexander	do	13	558	*C. quinquefasciatus*	11	do	1		Oct. 5, 1924	1	18			
A-20	do	do	18	551	*A. aegypti*	2	Richards B-3	1	do	Oct. 23, 1924	2	36	+	Oct. 29, 1924	5.75
				552	do	----	Reed B-1								
C-10	Squires	Nov. 11, 1924	9½	695	*C. quinquefasciatus*	56	Alexander A-20	1	Oct. 29, 1924	Nov. 20, 1924	16	22	—		
A-53	do	do	10	694	*A. aegypti*	15	do	1	do	Nov. 30, 1924	13	31	+	Dec. 5, 1924	5
C-11	White	do	15	719	*C. quinquefasciatus*	46	Kibbe A-28	1	Nov. 8, 1924	Nov. 26, 1924	12	18	—		
A-54	do	do	10	717	*A. aegypti*	31	do	1	do	Dec. 6, 1924	30	28	+	Dec. 13, 1924	7
C-12	Currier	Nov. 10, 1924	16	720	*C. quinquefasciatus*	43	Sparks A-27	1	do	Nov. 26, 1924	13	18	—		
A-55	do	do	10	718	*A. aegypti*	33	do	1	do	Dec. 6, 1924	30	28	+	Dec. 11, 1924	5

the egg of the infected mosquito (1905, 1906) and the importance of this matter from the standpoint of the prevention of dengue, information was sought experimentally on this point. The plan adopted in making this series of experiments was as follows:

Eggs were collected from the lots laid by *Aëdes* that had been proved to be infected. In order to be certain of this the mosquitoes that had been applied to a dengue patient on the first day of his disease were, after an adequate period (twelve or more days), allowed to bite a new and presumably susceptible volunteer. On the fourth and fifth days after this second biting the females laid eggs; these were collected and hatched. If the volunteer, bitten after the mosquitoes had become infective, came down with dengue, the infectivity of the mosquitoes that laid the eggs was considered to be demonstrated. The mosquitoes bred from such eggs were the ones used in this series of experiments. In some instances, the females that were the source of the eggs were permitted to bite first-day experimental cases a second time, the purpose being to infect them doubly and thus increase, if possible, the likelihood of transfer of the dengue virus to the eggs laid subsequent to such feedings. The adult *Aëdes* bred from such eggs were preserved in separate mixed lots and were held for from three to forty-two days prior to their experimental use. During this period they were fed solely on an aqueous solution of sugar.

They were allowed to take blood from three experimental subjects. A period of eight days was allowed to elapse before the various lots were again applied to the same patient. After a further period of eight days the negative results were controlled by exposing each of the volunteers to the lots of *Aëdes* known to be infected and most of which had actually caused dengue. In one instance a still further control was made with dengue-infected blood. The results are summarized in Table 8.

Three volunteers were used in this series, and the initial experiments with *Aëdes* bred from the eggs of infected females were entirely negative.

When the negative results were controlled by exposure of the subjects to the lots of infected *Aëdes* two of them promptly came down with dengue. In the third case (Davis) an attempt was made to infect with two lots of *Aëdes* (763 and 769) from which were bred many of the insects used in the initial experiments. There were only five females remaining in these two lots and, notwithstanding the fact that they were applied on two occasions

TABLE 8.—*Experimental data indicating that hereditary transmission of the dengue virus does not occur.*

Experimental subject.				Mosquitoes used in experiment.		Infection of experimental mosquitoes.		
						Name and experimental case No. of volunteer used—		
Experimental case No.	Volunteer.	Admitted to experimental ward—	Days in isolation or since bitten by mosquitoes.	Serial No. of lot.	Number in lot.	To infect mosquitoes from which eggs were secured to breed the *Aëdes* used in this experiment.	To infect mosquitoes.	Day of dengue in patient.
1	2	3	4	5	6	7	8	9
A-106	Hawkins	Feb. 14, 1925	8	871	24	Squires A-53		
A-113	do	do	8	871	22	do		
A-117	do	do	8	893	13		Capello A-100	1.00
				800	4		Hughes A-65	1.25
A-108	Shaver	do	8	870	11	Riggs Peterson		
A-115	do	do	8	872	10	Kibbe		
				870	11	Riggs Peterson		
A-119	do	do	8	872	9	Kibbe		
				900	24		Duncan A-101	1.00
				801	8		Wach A-69	0.75
A-107	Davis	do	8	813	5	Dembowski		
				815	5	Squires		
A-114	do	do	8	813	2	Dembowski		
				815	4	Squires		
A-118	do	do	8	763	3		Squires	0.25
				769	2		Riggs	2.25
B-15	do	do	7				Hawkins	2.00

Experimental case No.	Infection of experimental mosquitoes.		Biting of volunteers by infected mosquitoes.				Results.		
	Inclusive dates mosquitoes emerged from eggs of infected *Aëdes*.	Mosquitoes were infected—	Volunteer was bitten—	Number of females that took blood.	Inclusive number of days since adults had emerged from eggs.	Days virus had remained in mosquitoes.	Positive (+) or negative (—).	Positive cases.	
								Initial symptoms appeared—	Incubation period in days.
1	10	11	12	13	14	15	16	17	18
A–106	Feb. 10–18, 1925		Feb. 22, 1925	23	4 to 12		—		
A–113	do.		Mar. 2, 1925	19	12 to 20		—		
A–117		Feb. 26, 1925	Mar. 10, 1925	13		12	+	Mar. 15, 1925	4.75
		Jan. 7, 1925		3		62			
A–108	Feb. 5–19, 1925		Feb. 22, 1925	11	3 to 17		—		
A–115	Jan. 28–Feb. 14, 1925			9	8 to 25		—		
	Feb. 5–19, 1925		Mar. 2, 1925	10	11 to 25				
A–119	Jan. 28–Feb. 14, 1925			8	16 to 33		+	Mar. 14, 1925	4.50
		Feb. 27, 1925	Mar. 10, 1925	23		11			
		Jan. 7, 1925		5		62			
A–107	Jan. 19–28, 1925		Feb. 22, 1925	2	25 to 34		—		
	Jan. 28–31, 1925			4	22 to 25				
A–114	Jan. 19–28, 1925		Mar. 2, 1925	0	33 to 42		—		
	Jan. 28–31, 1925			4	30 to 33				
A–118		Dec. 5, 1924	Mar. 10, 1925	1		95	—		
		Dec. 9, 1924		1		91			
B–15		[a] Mar. 16, 1925	[b] Mar. 16, 1925	(c)		(d)	+	Mar. 22, 1925	6.0

[a] Date blood withdrawn from Hawkins.

[b] Date Davis was injected with blood.

[c] Amount of blood used, 0.5 cc.

[d] Time interval between withdrawal and injection of blood, ten minutes.

on the same day (March 10, 1925), only two would take blood. The lots, when used, were rather old (ninety-one and ninety-five days subsequent to their infection) and it was noted that, though all endeavored to take blood, only two succeeded in piercing the skin; the inference is that old age had resulted in degenerative changes of such a nature that the proboscis could not be inserted.

Conclusion.—We believe the conclusion is justified that the virus of dengue fever does not pass from the infected *Aëdes* through its eggs to the next succeeding generation.

MISCELLANEOUS OBSERVATIONS

The major phases of our experimental work and the conclusions drawn therefrom have been reported in preceding pages.

Other observations of interest and value were made during the course of the experiments, and the more important of these will be discussed in the following paragraphs.

Period of incubation in man.—As has been stated elsewhere, the criteria adopted for fixing the time of onset consisted of a combination of subjective symptoms plus elevation of temperature that thereafter continued above the normal range. The use of these factors as a basis enabled us to fix the incubation period fairly accurately. In some instances, in which the onset was sudden, subjective symptoms preceded sustained elevation of temperature by a few hours, but temperature above the normal constituted the determining factor.

The incubation periods in the forty-seven experimental cases are recorded in Table 2, column 16, page 97, and are summarized in Table 9.

TABLE 9.—*Incubation period in forty-seven cases of experimental dengue.*

Period.	Absolute numbers.	Per cent of total.	Per cent of total by groups.
Beginning of fourth to beginning of fifth day	11	23	89
Beginning of fifth to beginning of sixth day	18	38	
Beginning of sixth to beginning of seventh day	7	15	
Beginning of seventh to beginning of eighth day	6	13	
Beginning of eighth to beginning of ninth day	3	6	11
Beginning of ninth to beginning of tenth day	1	2	
Beginning of tenth to beginning of eleventh day	1	2	
Total	47		

In 61 per cent of the forty-seven experimental cases the incubation period falls between the beginning of the fourth and beginning of the sixth day after experimental biting. In forty-two (89 per cent) of the forty-seven cases, incubation ranged from four to seven days, inclusive. In three instances (6 per cent) the onset was delayed to the eighth day. In one case (Watkins A-40), the onset was fixed as being on the ninth day (9.25 days), but it is known that he had some suggestive subjective symptoms for a day or so prior to definite onset. In one other case (St. Hill A-60) the onset was at the beginning of the tenth day, though the patient began to have slight subjective symptoms two or three hours prior to that time.

Conclusion.—The incubation period of dengue fever in nonimmunes under the natural conditions encountered in the Philippines usually is from four to seven days, inclusive, exceptionally extending to the tenth day after infective biting. As will be pointed out in the section of this report dealing with immunity, the onset of symptoms may be delayed until the eleventh day in the case of individuals who are partially immune.

Immunity.—The experiments relating to immunity are discussed elsewhere in this series of reports, and the only reason for referring to them in this connection is to emphasize the fact that in doing experimental work with diseases transmitted by insects in an area of endemicity, immune individuals are quite likely to be encountered, and negative experiments that theoretically should have been positive are frequently explicable on this basis.

Notwithstanding the fact that the volunteers used by us in our early experiments were carefully selected with a view to the exclusion of those who had had previous attacks of dengue, we encountered four individuals (Wolde, Smith, Ptak, and Thomas) in whom we could not induce an attack of dengue by repeated exposure to lots of *Aëdes* known to have been infected; nor could we produce it in three of the four by subcutaneous inoculations of blood containing the living dengue virus.

It also is desirable to refer to the fact that three of the first six experimental subjects (Chambers, McNevin, and Hartman) also gave negative results with mosquitoes and with blood; but, in view of our present knowledge regarding the mechanism of transmission, we have discarded these cases as proving nothing, for the following reasons: The virus had not remained in the *Aëdes* long enough for them to have become infective; the *Aëdes* may have failed to pick up the virus from the dengue patients to which they were exposed (late stages of the disease); or it is possible that the blood used in the final control experiments did not contain the living dengue virus (it was taken from patients in the late stages of dengue). One of these volunteers (Chambers), in March, 1925, was given an injection of blood containing the dengue virus and he came down with an attack of dengue.

Altogether, sixty-four men were used in our mosquito transmission experiments and in this series we encountered six individuals (Wolde, Smith, Ptak, Thomas, Wilson, and Carroll) who proved to be entirely refractory even with control experiments. Three additional individuals (Chambers, McNevin, and Hartman) also seemed to be refractory, but they were eliminated from consideration for the reasons stated above. Whether or not Wilson and Carroll may have had mild attacks is discussed in the clinical section. There is some reason to believe they had.

Strains of the virus used.—The strains of virus used by us were obtained from two sources; one from a patient in Sternberg General Hospital, United States Army (Collins), on the third day of the attack, and one from a member of the board (Siler), on the first day of dengue. There is no doubt in our minds that the two strains were identical and that the disease in the latter instance was acquired through laboratory infection. This inference is based on the following facts: During the early part of October, 1924, the type of netting used on some of the mosquito storage cages had too large a mesh, and a few infected *Aëdes* were checked short; furthermore, some of our experimental cases came down with dengue on the same day that the member of the board came down (October 16, 1924).

The first strain (from Collins) was transferred to three volunteers by subcutaneous inoculations of blood, and from the volunteers so infected we obtained the strain that was used in producing dengue experimentally in forty-one individuals.

The second strain (from Siler) was transferred directly to *Aëdes* by exposing them to the patient during the first day of the disease, and produced experimental dengue in six volunteers, after which it was discarded.

The passage of these two strains from man to mosquito through successive generations is indicated in fig. 2.

The strain of the virus obtained from the member of the board was used for infecting purposes through three generations only and revealed nothing of particular interest.

The strain obtained from patient Collins was passed successively from human subjects through mosquitoes and back to man for six generations of mosquitoes. The clinical types of dengue observed in the cases produced experimentally by this strain

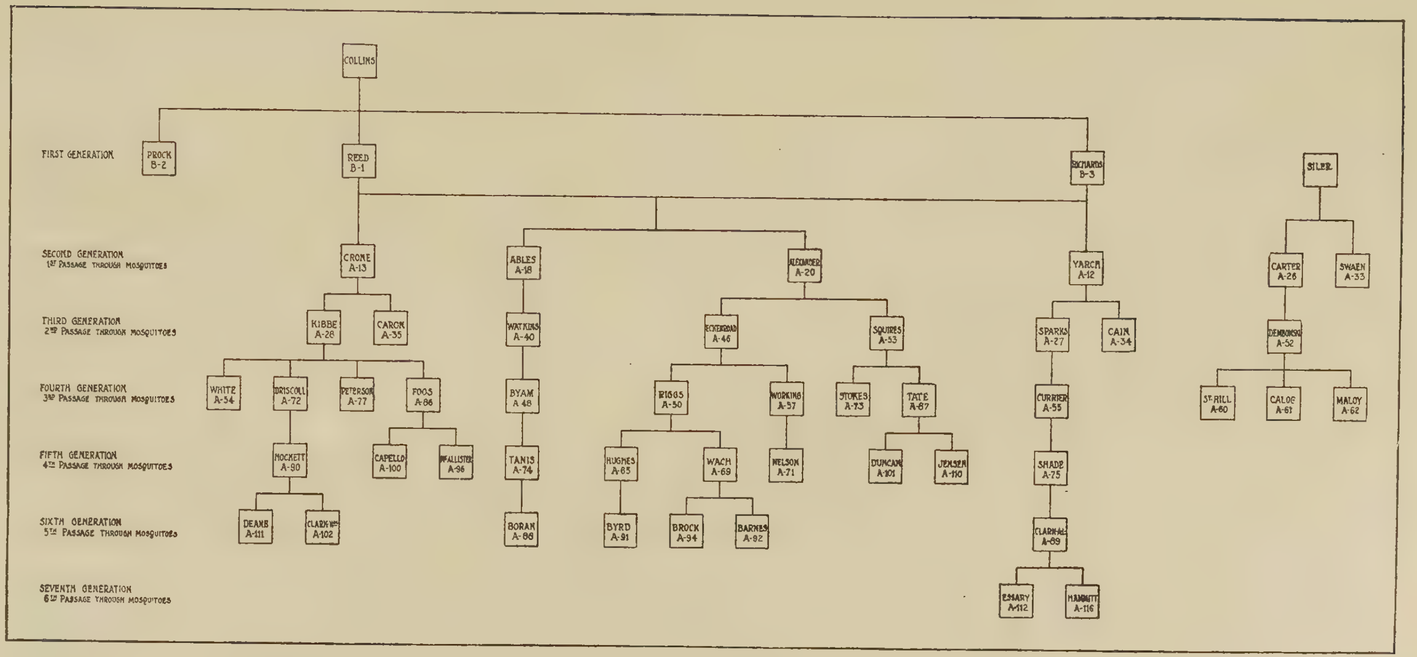


FIG. 2. Chart indicating passage of strains of the dengue virus from man to mosquito through successive generations.

are discussed in detail elsewhere, but it is pertinent to this discussion to state that every conceivable clinical type of dengue was encountered. That the virus did not suffer attenuation by successive passage through the mosquitoes is evidenced by the fact that Clark (A. L.) A–89, and Deane A–111, were infected by *Aëdes* representative of the fifth passage of the virus through mosquitoes, and the attacks of dengue from which they suffered were as severe as any that have ever been observed by us. On the other hand, it cannot be claimed that there was evidence of any increase of virulence as a result of continuous passage of the virus alternately through man and mosquito.

Number of Aëdes used in individual experiments.—We were aware of the fact that, in demonstrating the mechanism of transmission of yellow fever, the Yellow Fever Commission of the Medical Department, United States Army, had used very limited numbers of mosquitoes in each experiment.

As the disease with which we were dealing was mild in character and without mortality, and since many factors relating to the mechanism of its transmission required solution, we considered it the better part of wisdom to use considerable numbers of infected mosquitoes in each experiment.

The numbers of infected *Aëdes* that actually took blood from the experimental subjects at the time of infecting them with dengue, in our forty-seven positive transmission experiments, are shown in Table 10.

TABLE 10.—*Showing numbers of infected Aëdes mosquitoes that took blood from experimental patients.*

Positive transmission experiments.	Number of *Aëdes* used to produce dengue.	Positive transmission experiments.	Number of *Aëdes* used to produce dengue.
2	2	3	16
1	3	1	18
4	4	1	19
2	5	1	20
4	6	1	22
4	7	1	23
1	8	2	25
2	9	1	28
4	10	2	30
1	11	1	34
2	12	1	36
4	13		
1	14	47	

In twenty-four, or 50 per cent, of the positive cases the nonimmunes were bitten by from two to ten infected mosquitoes.

In two instances dengue was produced experimentally by the bites of two *Aëdes.*

Three or four attempts were made to transfer the virus with a single infected *Aëdes* but, unfortunately, the subjects used were proven subsequently to be immune and the demonstration of this point was not considered to be of sufficient practical or even theoretical importance to justify the use of military personnel solely for this purpose.

Time interval allowed for preliminary isolation and interval between transmission experiments.—In all experiments, except one, the period of preliminary observation as well as the time interval between transmission experiments was not less than eight days and in no case exceeded eighteen days. In two-thirds (thirty-one) of the forty-seven successful transmissions the interval was from eight to eleven days, inclusive; in fifteen others it extended from twelve to eighteen days, inclusive. In one instance (Dembowski A-50) the interval was shortened to seven days. The facts in this case are as follows:

He had been exposed previously to three lots of mosquitoes with negative results. He was then exposed to a lot of *Aëdes* that had been infected eleven days previously; since the results were negative after a period of seven days he was rebitten by the same *Aëdes;* the virus had at this time remained in the mosquitoes eighteen days. Seven and a quarter days after the last exposure he came down with an attack of dengue. Our interpretation of this series of transmission experiments was that the infection was due to the last exposure to *Aëdes.*

In 89 per cent of the forty-seven positive transmissions the incubation period of the disease was from four to six days, inclusive, and in no instance was it delayed beyond the beginning of the tenth day. A minimum period of eight days for isolation and to cover the interval between transmission experiments, plus a minimum allowance of four days for incubation of the virus in man, assured us of a minimum allowance of twelve days to cover any possible delayed incubation period in man. The maximum incubation period (one case) observed in the forty-seven cases constituting this series did not exceed ten days, and the interpretation of the results, in our opinion, is not open to criticism.

Transmission with blood.—As the investigations made by Ashburn and Craig in Manila (1907) and by Cleland, Bradley, and McDonald in Australia (1916, 1917) were complete and thorough, no special work was done by us on this phase of the problem. Transmission experiments with virus blood were made by us for two purposes only—to demonstrate immunity and to obtain a strain of the virus.

The results of the immunity experiments are recorded elsewhere in these reports. In six instances subcutaneous inoculations of from 0.5 to 1 cubic centimeter of virus blood were made as final negative controls. In three other instances (Reed B–1, Prock B–2, and Richards B–3), virus blood was injected. All three men came down with dengue, and it was from these three experimental cases, all inoculated with the same strain of the virus, that we obtained the strain used in forty-one of our positive mosquito-transmission experiments. The incubation period of the blood-transmission cases was from six and one-half to seven and one-quarter days (6.5, 6.75, and 7.25 days).

DISCUSSION

A brief general discussion of these experiments is desirable on account of the striking parallelism between the mechanism of transmission of dengue and that of yellow fever. Both are transmitted by the same species of mosquito, the patient in both is infective to the mosquito during the first three days of his illness, the development period of the virus in the mosquito is the same for both, and in both instances the infected mosquito retains its infectivity throughout life. The fundamental epidemiological characteristics of both diseases are very similar. Both are due to filtrable viruses which are present in the peripheral blood stream of the patient, and both diseases can be produced in the susceptible individual by injection of the virus-containing blood or its filtrate; clinically there are many points of resemblance between mild cases of yellow fever and typical cases of dengue, and their differentiation may be impossible; the first stage—that of congestion—is practically identical in the two diseases; in both diseases leucopenia with increase of lymphocytes occurs; both confer more or less immunity and, whereas that resulting from yellow fever is commonly believed to be lasting, it may depend, in part at least, upon the degree to which the immunizing functions of the body are stimulated.

The epidemiological factors common to both diseases summarized briefly above suggest the possibility that the living organisms causing both may fall into the same group, and it is with these facts in mind that the results of the experiments will be discussed.

Transmitting agent.—Considering first the insect transmitter, it has been suggested that mosquitoes other than *Aëdes* (*Stegomyia*) *aegypti* Linnæus may be vectors of dengue; namely, *Culex quinquefasciatus* Say. We feel that the evidence obtained in our experimental work quite definitely excludes *C. quinquefasciatus.* With regard to other species, particularly species of *Aëdes* other than *aegypti,* the statement is justified that their small number makes impossible their importance in the transmission of dengue in and about Manila.

All available evidence indicates that both yellow fever and dengue are transmitted solely by *Aëdes aegypti,* and epidemiological evidence seems at present so strong as to make it doubtful if any other species will ever be found important in the spread of dengue.

Incubation period of the virus in the mosquito.—It was only from the eleventh day subsequent to their infection that we were able to produce dengue through the bites of infected *Aëdes,* and the same phenomenon has been observed for yellow fever. The evidence we have obtained suggests that, even on the eleventh day after the infection of the mosquito, the virus may not have developed sufficiently to make it invariably capable of producing the disease. It is probable that not until the twelfth to the fourteenth day after its passage into the mosquito does the virus attain a high degree of invasive power. It has been demonstrated that there is a similar threshold of uncertainty just previous to the time when the mosquito carrier of the virus of yellow fever becomes highly infective; the evidence indicates that the chronology of development of the two viruses in the mosquito is identical.

Period of infectivity of the patient.—In dengue, as was found to be the case in yellow fever, the patient is infective to mosquitoes during the first three days of his illness. Since dengue fever is very mild, as compared with yellow fever, it was possible for us to investigate somewhat more fully this phase of the problem. We found that the individual with dengue is infective to *Aëdes* during the late prodromal stages of the disease several hours prior to the appearance of definite symptoms. The evidence indicates very definitely, as would be expected, that the patient is most infective to the mosquito during the first day of his illness and by the end of the third day the virus has disappeared from the peripheral circulation or has become so attenuated or diluted that the *Aëdes* does not become infected. In severe and somewhat prolonged cases of dengue, it is conceivable that even on the fourth day of the attack the virus may be present in the peripheral circulation in sufficient concentration to insure its transfer to the *Aëdes*, but we believe that this occurs with such rarity as to be of no practical importance.

Were it feasible to investigate the infective period of the patient with yellow fever, it is very probable that like results would be obtained.

Length of time infected Aëdes aegypti remain infective.—Once an *Aëdes aegypti* has taken the virus of dengue into its body and become capable of transmitting it, this quality usually is retained throughout the remainder of its life. A like characteristic has been demonstrated to apply to yellow fever.

Hereditary transmission of the dengue virus in the mosquito.—Information concerning this factor in transmission is of considerable importance, and we feel that the experimental evidence incorporated in this report justifies the statement that hereditary transmission of the virus through the egg of the infected mosquito does not occur.

Marchoux and Simond (1905) report that they succeeded, in one instance, in producing yellow fever with mosquitoes bred from the eggs of infected mosquitoes.

We have reviewed their report and, while the experimental evidence on which their conclusions are based is very suggestive, we believe that some of the arguments presented by them in support of their conclusions are not well founded, and also that the subject of the one positive result reported might possibly have acquired the disease from some other source.

One of the principal reasons given by these investigators for undertaking the experiments in transmission through eggs was their assumption that only in this way was it possible to explain the reappearance of yellow fever in certain localities at certain times. We believe that it is just as reasonable, and in fact more so, to assume that the disease is kept going in areas of endemicity through its occurrence in mild form in children and through mild "missed" cases in other nonimmunes concerning whom the health authorities have no information. We are quite confident that dengue is kept going in its endemic centers, certainly in Manila, in this manner.

It is now known that the eggs of *Aëdes aegypti* are very resistant to an unfavorable environment and will hatch subsequent to storage for several months in a dry place. Experiments made by us show that eggs will hatch after storage for at least ten weeks at low temperatures. The records of epidemics of yellow fever in geographical areas in which low atmospheric pressure obtains throughout the winter months evidence the fact that epidemics cease with the advent of cold weather and do not recur the following hot season unless the disease is re-imported, even though a large proportion of the population still is nonimmune. These factors do not favor the hypothesis of transmission through the egg.

Coming now to the experimental case reported, there seems to be no doubt that it was a mild case of yellow fever, though without some of the characteristic symptoms—albuminuria, jaundice, and black vomit.

The symptomatology recorded is strikingly similar to that frequently observed in cases of dengue, but this possibility was fairly definitely excluded in the control biting experiments with infected *Aëdes,* though not completely so, insofar as we can determine from the report. (The mosquitoes infected from cases of yellow fever and used in the control experiments never actually produced a case of yellow fever.) There is the remote possibility, therefore, that the investigators were dealing with dengue rather than with yellow fever, but this interpretation is in all probability not the correct one.

As the number of potentially infected mosquitoes used in the two control experiments was small (two in the one instance and one in the other) there remains the possibility that the insects used were incapable of transmitting the virus. Failures of this nature have been reported in experimental yellow-fever work, and we had similar experiences in our dengue-transmission experiments.

Assuming that the subject of the experiment actually had a mild attack of yellow fever, it is possible (or even probable) that the infection was acquired from mosquitoes other than those actually used by the experimenters. It seems to us that the environment in which the experimental subject lived, in so far as protection from wild infected mosquitoes is concerned, is subject to serious criticism. The subject remained in a laboratory to which his meals were brought, and the inference that one would draw from reading the report—which we concede may possibly be an incorrect one—is that work was being carried on in the laboratory during the period of his confinement therein, that infected mosquitoes were kept in the same room, and that there must have been occasion to open the doors leading to the room many times each day.

There is the possibility, therefore, that an infected mosquito gained entrance or that an infected mosquito inside the room accidentally escaped and that the infection was acquired in this manner. Even though the incubation period fitted in so perfectly, we believe that one isolated case of this kind should not be given undue weight.

We feel, therefore, that considering the obvious flaws in the experiment itself and the fact that this one case constitutes the unique support for the supposition that the yellow-fever virus may pass through the mosquito hereditarily, there is here no basis for discussing the transfer of the yellow-fever virus through the egg of the *Aëdes*. Our completely negative results in attempts to cause the dengue virus to go through the egg of this mosquito to the succeeding generation lead us to believe that such inheritance does not occur in the case of dengue.

SUMMARY

This report presents in detail a somewhat extensive series of experiments relating to the transmission of dengue by mosquitoes. These investigations have been pursued by the United States Army Medical Department Research Board at the Bureau of Science and at the Sternberg General Hospital in Manila.

Part I considers the plans and arrangements made and the preliminary work done in preparation for the actual experimental work. Part II concerns itself with the various sets of experiments made, the results obtained, and the conclusions drawn therefrom.

Part I.—The preliminary arrangements had for their basis the scope of the work contemplated, which covered the following points: Confirmation of the reported transmission of dengue by the mosquito *Aëdes* (*Stegomyia*) *aegypti;* incrimination or elimination of *Culex quinquefasciatus* as a transmitter; and investigation of the exact mechanism of transmission by mosquitoes.

Only two species of mosquitoes were used in the transmission experiments, *A. aegypti* and *C. quinquefasciatus*, and the reasons for so doing are explained. The arguments presented indicate that no species other than these two are concerned in the transmission of dengue in Manila.

All the mosquitoes used in the experiments were bred from the egg. A period of approximately four months was spent in testing various food substances that might be suitable for the propagation of larvæ and in developing and perfecting a routine breeding technic. Normal horse serum was finally selected for this purpose, and formalin 1: 2,500 to 1: 5,000) was added to inhibit bacterial growth. In the later stages of the experimental work many experiments were made in breeding larvæ in solutions of tap water to which slices of ripe banana had been added, and this type of food was found to be superior to blood serum. In work of a similar nature the use of banana as food for larvæ is recommended for both *A. aegypti* and *C. quinquefasciatus.*

All reserve stocks of adult mosquitoes were fed on aqueous solutions of sugar, and this type of food proved to be satisfactory in all respects.

As a rule *A. aegypti* mosquitoes were used experimentally for initial biting from two to seven days after emergence, and *C. quinquefasciatus* mosquitoes were not more than five days old when first fed on blood.

The exact number of mosquitoes, as well as the species used in each experiment, was always known, and the biting of human beings was always under control and exclusively at the will of the board.

After the infecting exposure to dengue patients of freshly bred mosquitoes, all not showing complete distention with blood were removed and killed. It was known, therefore, that all mosquitoes used subsequently to determine their infectivity for volunteers had been potentially infected.

Freshly bred *Aëdes aegypti* would not bite freely on the day of their emergence, but after they had taken food (solution of sugar) and had been fertilized, they took blood freely—usually 100 per cent of them.

Aëdes aegypti in the Philippine Islands bite freely at any time during the day, and night biting, though unusual, also is observed. The biting habits of *C. quinquefasciatus* were found to be erratic; they would take blood only at night and, even under the most favorable conditions, but a relatively small proportion would do so.

The experimental subjects consisted of military personnel that proffered their services voluntarily. Sixty-four men were used. The volunteers were specially selected and in general met certain basic requirements—freedom from disease, including syphilis; short service in the Philippines; and nonimmunity to dengue.

The experiments were made in a specially prepared ward in a large military hospital in Manila, and extraordinary precautions were taken to exclude mosquitoes. The ward was administered by specially selected personnel, one of the most important functions of whom was the detection and destruction of mosquitoes that might possibly gain entrance to the ward or its vestibules.

Part II.—The transmission experiments presented in this report include a total of fourteen injections of virus blood, of which five were positive; one hundred eleven biting experiments with *Aëdes aegypti,* of which forty-seven were positive; and seven biting experiments with *Culex quinquefasciatus,* all of which gave negative results. Among the sixty-four volunteers used, dengue was produced experimentally in fifty-two instances (81 per cent).

In the conduct of the experiments, the general policy was adopted, in each series, of using successively all available methods for producing the disease—biting, followed in many instances by repetition, and this followed in turn by injections of virus blood. All negative results were adequately controlled—biting by mosquitoes known to be infective or by injections of virus blood.

Previous reports of the transmission of dengue by *Aëdes aegypti* were confirmed—forty-seven positive results.

Experiments were made with eight volunteers to fix the incubation period of the virus in the mosquito, and it was found that the mosquito did not become infective until the eleventh day after its infection. The evidence obtained indicates that even on the eleventh day after their infection *Aëdes aegypti* may be incapable of transmitting the virus. The limits of incubation of the virus in the mosquito apparently are from the eleventh to the fourteenth day.

Experiments were made with twenty-one volunteers to determine the stages during which dengue patients are infective to *Aëdes aegypti.* The results obtained indicate that the patient is infective to mosquitoes during the first three days of the disease, but that on the third day of an attack the mosquito will frequently fail to pick up the virus. It is demonstrated, furthermore, that individuals in the late prodromal stages of dengue—six to eighteen hours prior to onset—are infective to *Aëdes.*

The experimental evidence obtained warrants the statement that once *Aëdes aegypti* becomes capable of transmitting the virus to human beings this characteristic is retained throughout the remainder of the mosquito's life. Experimental dengue was produced in three volunteers with mosquitoes that had been infected, respectively, sixty-two, sixty-six, and seventy-five days previously.

Endeavors were made to infect seven volunteers with potentially infected *Culex quinquefasciatus* (*C. fatigans*), and all such experiments were entirely negative. The volunteers were then bitten by *Aëdes aegypti,* infected from the same sources and on the same day as the *C. quinquefasciatus* used in the previous experiments, and all came down with dengue. The conclusion is drawn that *Culex quinquefasciatus* does not transmit dengue.

Three volunteers were used to ascertain the possibility of the hereditary transmission of the virus in the mosquito. The results obtained were entirely negative. When the experimental subjects were subjected to control experiments all three developed dengue. The evidence suggests very definitely that the virus of dengue fever is not carried from infected *Aëdes aegypti* through its eggs to the next succeeding generation.

The incubation period of the disease in the forty-seven experimental cases varied from four to ten days, inclusive. For all practical purposes the incubation period may be considered as being from four to six days, inclusive, as it fell within that period in 89 per cent of the experimental cases reported.

In forty-one of the forty-seven cases of dengue experimentally produced the virus was derived from the same strain, and this strain was passed from man to mosquito and back to man through six generations. There was no evidence that the virus suffered attenuation or that its virulence was increased as a result of continuous alternate passage through man and mosquito.

The numbers of potentially infected *Aëdes* that took blood for infecting purposes in the forty-seven positive cases varied from two to thirty-six, and 50 per cent of the positive cases were bitten by from two to ten potentially infected mosquitoes.

The preliminary periods of isolation and the time interval between biting experiments, with one exception, were not less than eight days and did not exceed eighteen days.

EPIDEMIOLOGY OF DENGUE IN THE PHILIPPINE ISLANDS

INTRODUCTION

The consideration of the epidemiology of dengue in the Philippines is limited mainly to the study of the manifestations of the disease among the foreign population, for the reason that it seldom affects the native in a recognizable form and is not a factor in mortality. Whether the native Filipino enjoys an absolute immunity to dengue or is protected by repeated reinfection from earliest childhood will be discussed in the consideration of the immunity conferred by an attack of the disease.

The fact that dengue is rarely a cause of death accounts for the lack of all statistics from civil sources as to its prevalence. Hence we have no definite knowledge of the extent to which it occurs in Caucasians of long residence in the Islands. The general opinion of physicians and of old residents is that when an American or European first arrives in the Philippines he will almost certainly contract dengue within his first year. Many of them suffer subsequent attacks, in general of diminishing severity, until after several years the attacks, if they occur at all, are so mild and so atypical that they may no longer be certainly recognized as dengue. Occasionally, after many years of such freedom, a typical form of the disease develops. It seems certain, therefore, though the opinion is not supported by any statistical evidence, that a considerable degree of immunity or tolerance is developed as the result of repeated attacks. The military establishment, however, furnishes large numbers of unprotected individuals annually, and only from the army are morbidity statistics available on which to estimate the prevalence and importance of the disease.

The statistical data presented herein were obtained from the Annual Reports of the Surgeon General of the United States Army, from reports on file in the Office of the Surgeon, Philippine Department, and from the retained records on file at the various stations.

DENGUE IN THE MILITARY ESTABLISHMENT

Although a nonfatal disease of short duration, dengue has shown itself in the past to be capable of seriously crippling a military command in the field, and for this reason the disease is well worth the attention of students of preventive medicine as applied to the military forces. Preventive medicine has accomplished notable results in the Philippines in the years of American occupation, and it may be pointed out here that the general morbidity and mortality rates of troops in the Islands have shown a marked decline, as practical methods of handling the problems involved have developed and been put into practice. The figures showing the tendency to improvement in sickness and death among the troops are given in Table 11 and are graphically presented in figs. 3 and 4.

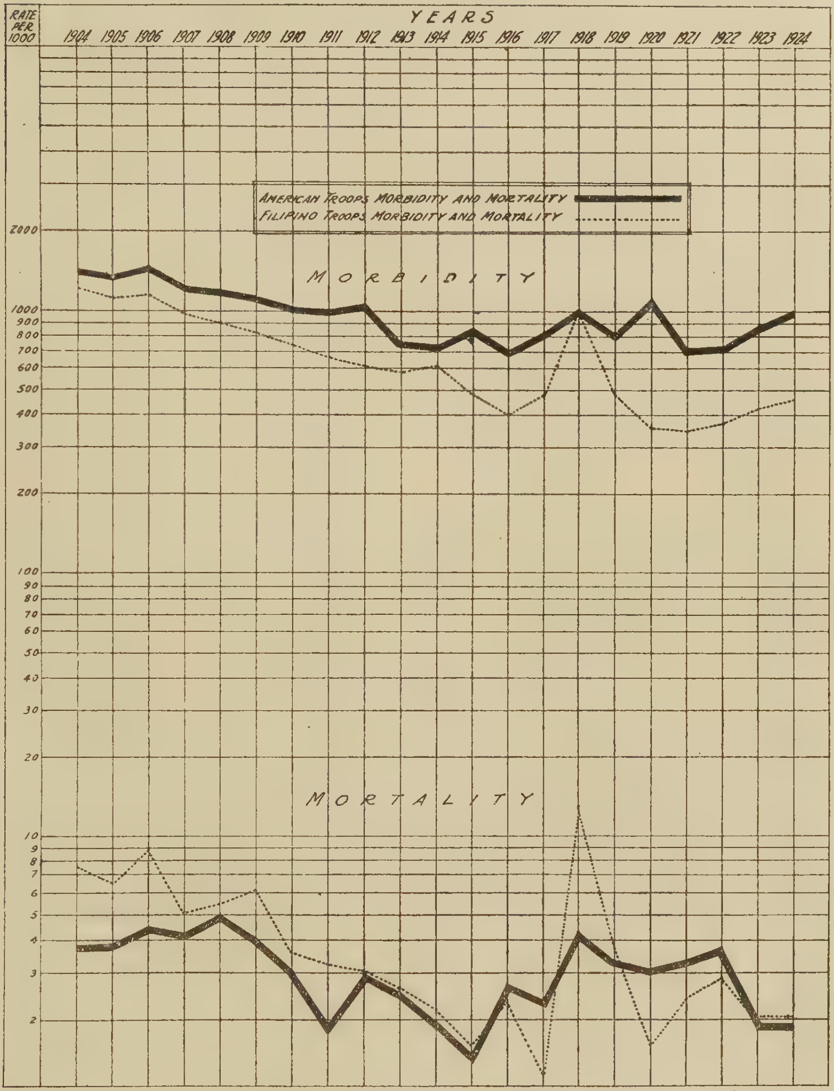


FIG. 3. Chart showing morbidity and mortality rates, disease only, American and Filipino troops (enlisted) on duty in the Philippine Islands.

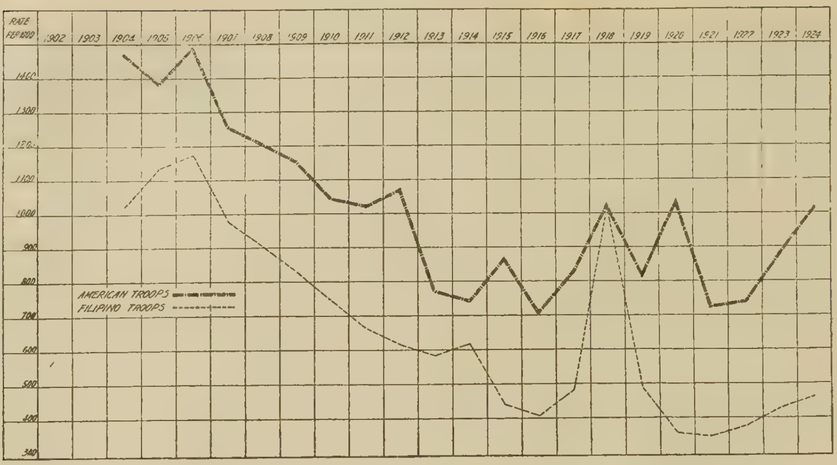


FIG. 4. Chart showing admissions, disease only, American and Filipino troops, Philippine Islands, 1904 to 1924, inclusive. Rate per 1,000.

TABLE 11.—*Admissions and deaths (disease only) among American and Filipino troops, Philippine Islands, 1904 to 1924, inclusive.*[a]

Year.	Strength.		Admissions, disease only, rate per 1,000.		Deaths, disease only, rate per 1,000.	
	American.	Filipino.	American.	Filipino.	American.	Filipino.
1904	11,996	4,610	1,473	1,023	3.83	7.59
1905	11,057	4,732	1,387	1,137	3.89	6.47
1906	12,380	4,759	1,494	1,175	4.52	8.93
1907	11,699	4,679	1,260	981	4.27	5.13
1908	11,971	5,085	1,208	908	5.03	5.57
1909	12,844	5,369	1,156	831	4.09	6.14
1910	12,277	5,093	1,048	748	3.08	3.58
1911	12,454	5,266	1,028	667	1.93	3.23
1912	12,357	5,407	1,075	617	2.99	3.09
1913	11,188	5,096	776	585	2.55	2.67
1914	10,253	5,020	747	618	1.99	2.19
1915	11,834	5,505	871	483	1.51	1.63
1916	11,580	5,579	714	407	2.76	2.33
1917	10,879	5,509	832	482	2.39	1.27
1918	7,381	5,982	1,030	1,023	4.34	13.21
1919	5,422	8,391	827	488	3.32	3.81
1920	10,574	7,295	1,042	357	3.12	1.65
1921	8,134	6,998	730	347	3.32	2.43
1922	5,628	6,549	743	377	3.73	2.90
1923	3,789	7,050	887	429	2.38	2.27
1924	4,869	7,072	1,019	462	2.05	1.97

[a] Figures from Annual Reports of the Surgeon General, United States Army.

The former chart (fig. 3), plotted on a logarithmic scale to allow comparison between the sickness and death rates, shows that from 1904 to 1916 there was a general decline in morbidity, and at a nearly uniform rate, as is evidenced by the fact that the rates for these years plot out in a nearly straight line. The decline in mortality roughly parallels that of morbidity. The improvement in both respects is more marked for Filipino troops than for Americans. Following 1916 the increasing morbidity and mortality associated with the evolution of the great influenza epidemic began to make itself felt, reaching a peak in 1918. Following this peak the mortality has again shown a tendency to decline, but the admission rate has in general maintained itself at a level slightly higher than before the influenza. This, however, is true only for American troops, as the chart shows that the rates during the later years continue relatively much lower for Filipinos than for the whites. The rates for admissions, disease only, for the two groups are arithmetically plotted in fig. 4. This chart shows even more definitely the lower rates in recent years.

The rates for dengue, on the contrary, show no such satisfactory trend. The morbidity statistics for dengue, 1902 to 1924, inclusive, are incorporated in Table 12 and presented graphically in figs. 5 and 6.

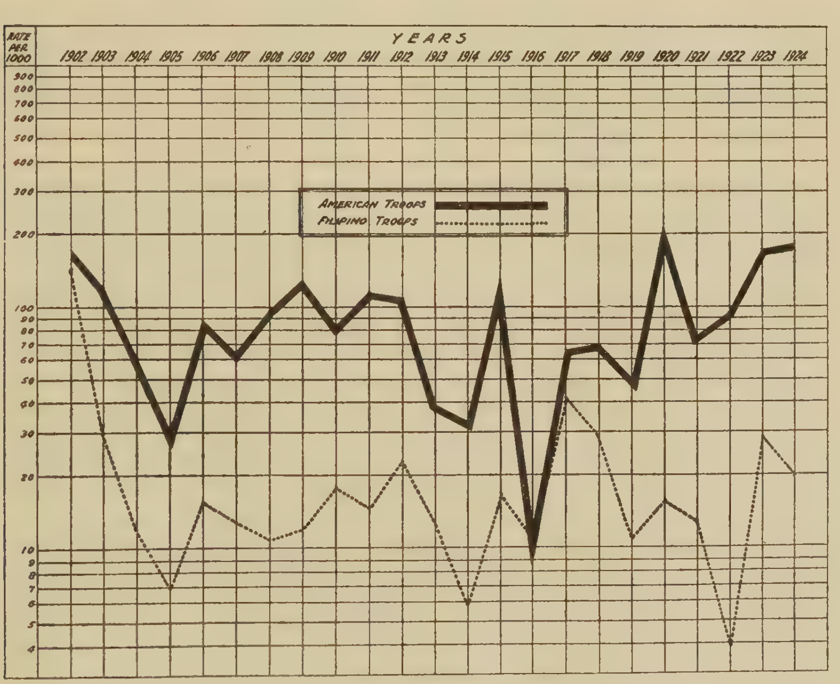


FIG. 5. Chart showing morbidity rates, dengue fever, United States military troops on duty in the Philippine Islands, 1902 to 1924, inclusive. Rates per 1,000 per annum for American and Filipino troops (enlisted only).

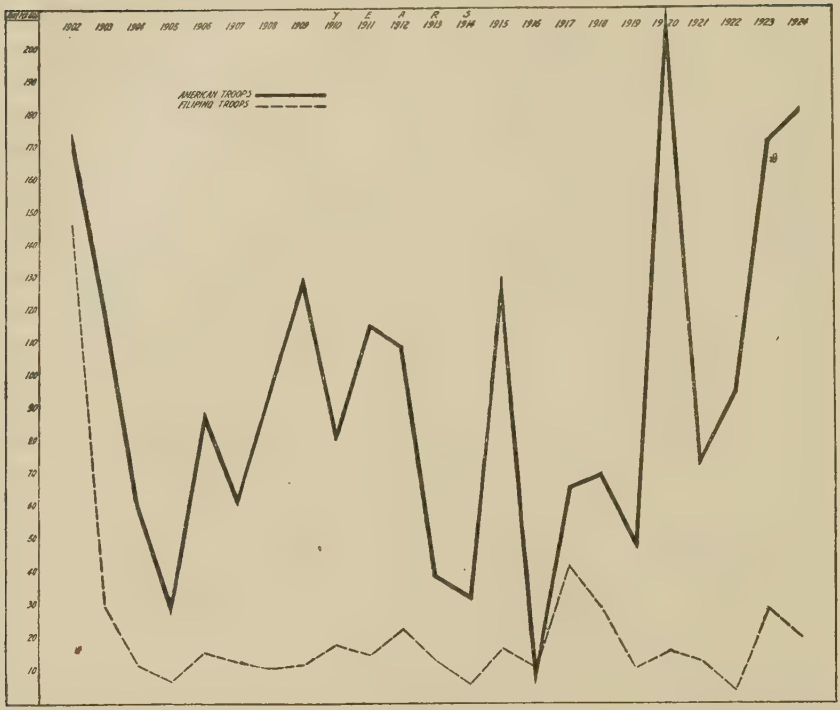


FIG. 6. Chart showing admission rates, dengue fever, military forces on duty in the Philippine Islands, 1902 to 1924, inclusive. Rates per 1,000 per annum for American troops (enlisted) and for Filipino troops (enlisted).

In the charts the rates for Filipino and American troops are plotted separately, showing the marked difference in rates between the two groups. Both colored and white troops are included as "American," because during the years when a sufficient number of colored troops were present in the Islands to make their rates of any value, no significant differences were shown between them and those of the white troops. In the interpretation of the data thus presented it is essential that two points be borne in mind. The first is that for a number of years the tour of duty in the Philippines for American troops has been limited to two years and for the past twenty years has not exceeded three years. But few exceptions have been made to this general rule and, practically speaking, there is a complete turnover of the American personnel every two years. This means, of course, that unprotected individuals are arriving from the United States on every transport, constantly adding nonimmunes to the military population, and this turnover naturally accounts for the unduly high rates in American troops.

TABLE 12.—*Admissions for dengue fever, 1902 to 1924, inclusive, Philippine Department.*[a]

Year.	American troops.			Filipino troops.		
	Strength.	Cases.	Rate per 1,000 per annum.	Strength.	Cases.	Rate per 1,000 per annum.
1902	32,942	5,763	175	4,826	710	147
1903	19,029	2,305	121	4,789	145	30
1904	11,996	728	61	4,610	46	12
1905	11,057	341	31	4,732	31	7
1906	12,380	1,099	89	4,759	74	16
1907	11,699	738	63	4,679	62	13
1908	11,971	1,162	97	5,085	56	11
1909	12,844	1,672	130	5,369	66	12
1910	12,277	1,017	83	5,093	92	18
1911	12,454	1,444	116	5,266	81	15
1912	12,357	1,346	109	5,407	125	23
1913	11,188	439	39	5,096	65	13
1914	10,253	340	33	5,020	28	6
1915	11,834	1,554	131	5,505	92	17
1916	11,580	142	12	5,579	62	11
1917	10,879	713	66	5,509	230	42
1918	7,381	513	70	5,982	174	29
1919	5,422	270	50	8,391	97	11
1920	10,574	2,248	213	7,295	119	16
1921	8,134	606	75	6,998	88	13
1922	5,628	535	95	6,549	25	4
1923	3,789	651	172	7,050	206	29
1924	4,869	885	182	7,072	143	20
Total	262,537	26,511	101	130,661	2,817	22
Average per annum for 23 years	1,141.5	1,153	101	5,681	122.5	22

[a] Figures from Annual Reports of the Surgeon General, United States Army.

The second factor to be borne in mind concerns diagnosis. The clinical manifestations of dengue are so variable, and in any epidemic there are likely to be so many mild cases, with rash evanescent or absent, that diagnosis becomes largely a matter of opinion. The result is that some medical officers report as dengue a much smaller proportion of their mild fever cases than do certain others, who are not quite so conservative. Analysis of the symptomatology exhibited by forty-one cases of dengue experimentally produced by us from a single strain of virus suggests that appreciable numbers of soldiers suffer attacks of so mild a nature that they never report themselves as sick. We therefore venture the opinion that if malaria and infections of the respiratory tract be excluded, most of the undetermined fevers of short duration (three to six days) encountered in American troops in the Philippines, especially in Manila and its immediate environs, are in reality dengue. It seems entirely probable, therefore, that the figures given in Table 12 and plotted in figs. 5 and 6 are a decided understatement of the incidence of this disease. They do, however, represent with some accuracy the number of cases of sufficient severity to cause loss of time from military duties. A series of twenty-five consecutive cases admitted to Sternberg General Hospital during the fall of 1924 showed an average stay in hospital of six and seven-tenths days. At this rate the average number of days lost per year for the last twenty-three years is 7,715, without taking into account the lack of efficiency for a variable period after return to duty. This figure represents the time lost per year from duties and training by American troops only. Considering the small number of troops in the Islands, it is seen that dengue is the cause of a serious economic loss to the Government, and any measures that might materially lessen its incidence are well worth taking.

As further evidence of the relative importance of dengue as a cause of disability among American troops the data presented in fig. 7 have been worked out. This chart shows the rates per thousand per annum for the three leading causes of admission to sick report for the years 1904 to 1924, inclusive, among American troops only. The figures were obtained from the Annual Reports of the Surgeon General. The three conditions leading to the highest morbidity rates during this period were found to be venereal diseases, malaria, and dengue. Venereal diseases during the entire period maintained first place in this list, except in the years 1920 and 1924, when the dengue rate rose above that for venereal diseases. The general trend of incidence of venereal diseases has been decidedly downward. This downward trend has been still more marked in the case of the malarial fevers, and of late years new cases of malaria practically do not occur except at one station, Camp Stotsenburg, where conditions are especially difficult to control. It is to be understood that these remarks apply to American troops only. During the earlier part of the occupation period dengue was distinctly secondary to malaria as a cause of sickness; but

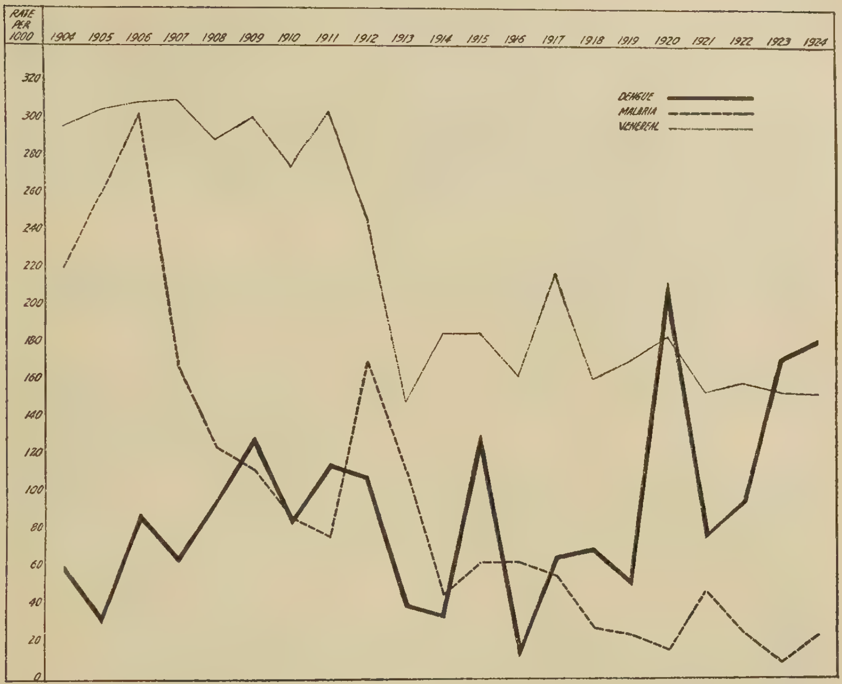


FIG. 7. Chart showing morbidity rates for dengue, venereal diseases, and malaria; American troops in the Philippine Islands, 1904 to 1924. Rates per 1,000 per annum.

of late years, with the gradual abandonment of many stations in the provinces and the concentration of a relatively larger proportion of the troops in Manila and its immediate environs, the relative position of the two has been reversed and the rates for dengue have markedly increased. While great variation in prevalence is noted from year to year, inspection of fig. 6 serves to convince one that dengue is increasing rather than diminishing among the military personnel. The low rates in 1913, 1914, and 1916 are striking. Table 13, giving the total numbers of troops in the Philippine Islands and the numbers stationed in Manila for the years 1904 to 1924, is interesting in this connection.

In 1916, but 960 American soldiers were in Manila; in 1919, but 695. Then, in 1920, the number was increased to 2,956—more than four times as many. The arrival of so many susceptible persons within so short a time could easily be responsible for the high rate in 1920.

The charts show strikingly the lesser degree of susceptibility of the Filipino soldier to this disease. During the years

1902 to 1924, inclusive, dengue attacked American troops with five times greater frequency than it did Filipino troops. We believe that the very low rates in Filipino organizations are the result of an acquired immunity or tolerance to the disease. The reasons for holding this view, as regards both the native soldier and the acclimatized white civilian, are stated in detail in the discussion of immunity to dengue.

TABLE 13.—*Total American troops in the Philippine Islands, showing number and percentage of troops in Manila, 1904 to 1924.*

Year.	American troops in the Philippine Islands.	American troops in Manila.	Percentage of American troops in the Philippine Islands who were stationed in Manila.
1904	13,881	2,936	21.1
1905	13,301	1,721	12.9
1906	14,156	990	7.0
1907	12,810	968	7.6
1908	13,134	1,018	7.8
1909	14,047	1,270	9.0
1910	13,461	1,236	9.2
1911	13,346	858	6.4
1912	14,226	1,453	10.2
1913	13,381	1,307	9.8
1914	12,552	1,064	8.5
1915	13,849	1,263	9.1
1916	13,486	960	7.2
1917	12,344	1,565	12.7
1918	8,351	1,218	14.5
1919	6,218	695	11.2
1920	12,480	2,956	23.6
1921	9,800	2,548	26.0
1922	7,007	1,842	26.3
1923	4,574	1,664	36.4
1924	4,829	1,687	34.9

DENGUE AT MILITARY STATIONS IN THE PHILIPPINES

The United States military forces, American and Filipino, in the Philippines are concentrated at seven stations.

The Post of Manila is situated in Manila itself, and its principal garrison is a regiment of American Infantry, Thirty-first United States Infantry (average strength, 1922, 1,094; 1923, 1,090; 1924, 1,101). The Sternberg General Hospital, United States Army, also is an integral part of this station (average strength, 1922, 144; 1923, 147; 1924, 171).

Fort William McKinley, one of the largest stations, is located 14 kilometers southeast of Manila (average strength, American troops, 1922, 247; 1923, 551; 1924, 851).

Fort Mills, another large station, is situated on Corregidor Island, in Manila Bay, 48 kilometers from Manila (average strength, American troops, 1922, 2,352; 1923, 1,057; 1924, 1,363).

Camp Stotsenburg, the remaining of the larger stations, is situated on Luzon Island, 91.2 kilometers north of Manila (average strength, American troops, 1922, 728; 1923, 238; 1924, 212).

Camp Nichols, a small station, is located on the outskirts of the city of Manila (average strength, American troops, 1922, 296; 1923, 398; 1924, 447).

Camp John Hay, which is located in the mountains of northern Luzon, has a small garrison; and Pettit Barracks, situated at Zamboanga, Mindanao, also is a small station, garrisoned by Filipino troops.

Our inquiry into the dengue situation at military stations was limited to its occurrence during the years 1922, 1923, and 1924, at five stations—Post of Manila (Thirty-first Infantry and Sternberg General Hospital), Fort William McKinley, Fort Mills, Camp Stotsenburg, and Camp Nichols—as for these stations only, during these years, were satisfactory statistics available.

The situation at Camp John Hay, in the Mountain Province, may be dismissed with the statement that dengue does not occur there. The camp is located in the mountains at approximately 1,500 meters elevation, and the natural environment is unfavorable to the propagation of *Aëdes aegypti.* Army regulations (Medical Department) have provided for many years that collections of mosquitoes be sent to the Army Medical Museum, Washington, D. C., at monthly intervals, from all army stations. We have been unable to find any record of *Aëdes aegypti* ever having been present in any of the collections sent from Camp John Hay to the Army Medical Museum.

Dengue has been reported from Pettit Barracks, but the garrison consists of native troops and the number of cases reported is so small that it may de dismissed from consideration.

The annual dengue morbidity rates for American troops for the five stations referred to are presented in fig. 8.

It is evident from fig. 8 that high morbidity rates from dengue are common in units serving in Manila (Thirty-first

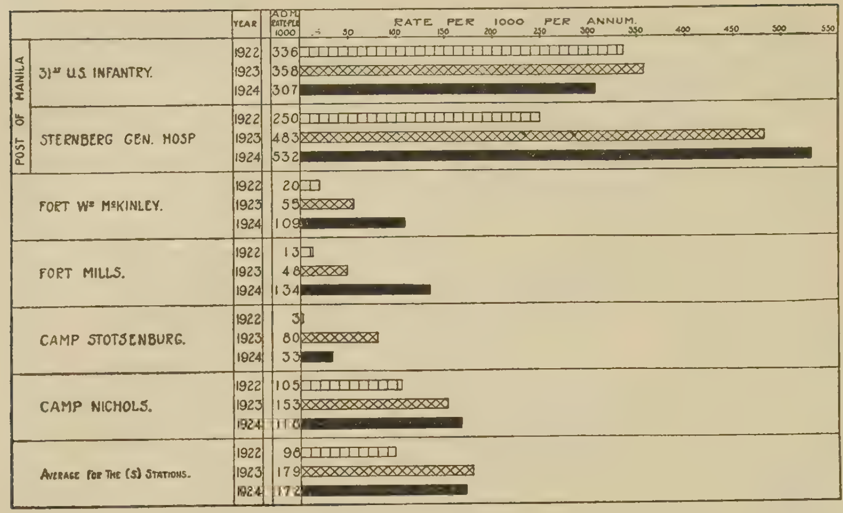


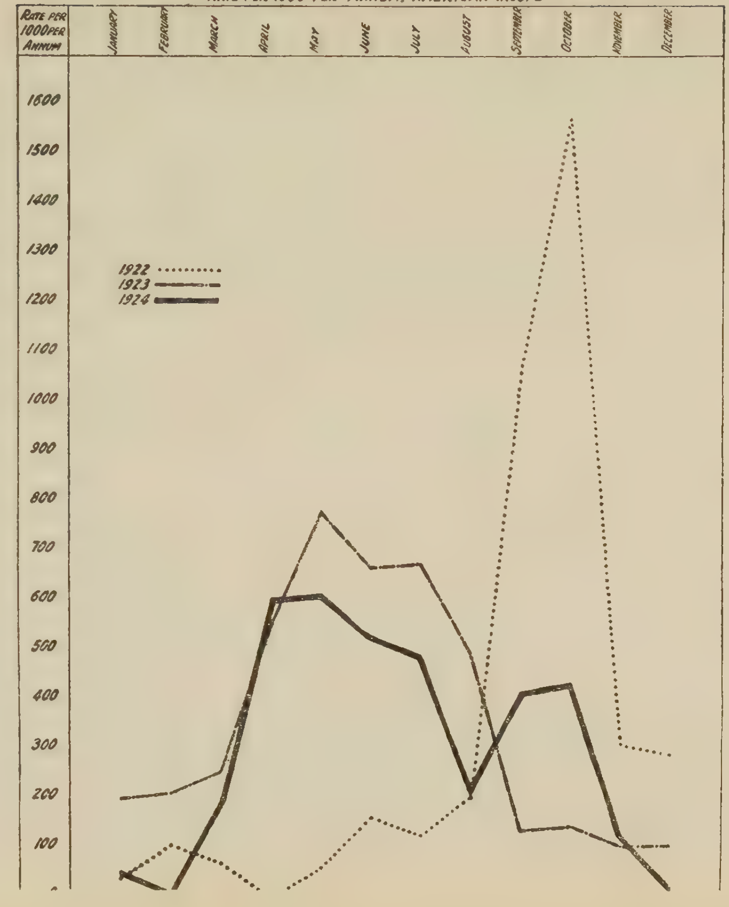
FIG. 8. Chart showing dengue rates at military stations in the Philippine Islands, 1922 to 1924. American troops only. Rates per 1,000 per annum.

United States Infantry and Medical Department, Sternberg General Hospital), and that these rates have remained consistently high for the three years 1922 to 1924. On the contrary, the rates at the three stations in the provinces, and even at Camp Nichols on the outskirts of Manila, have been low, though there have been progressive and somewhat marked increases at Fort William McKinley and Fort Mills.

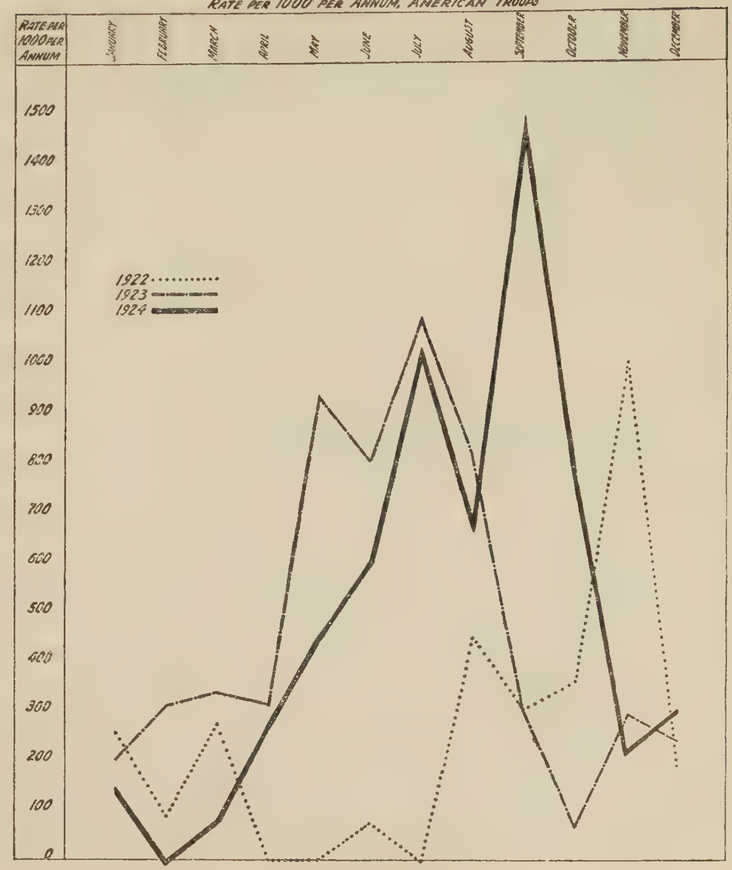
The dengue situation at these stations is best visualized by referring to Tables 14 to 19, inclusive, and to fig. 9.

At Fort William McKinley and Fort Mills a few cases of dengue occur throughout each dengue season, most of which doubtless are acquired during visits to Manila, and occasional small explosive outbreaks occur which usually are confined to one or two organizations. When such localized outbreaks do occur inspections demonstrate that the organization has become lax in the application of mosquito-control measures.

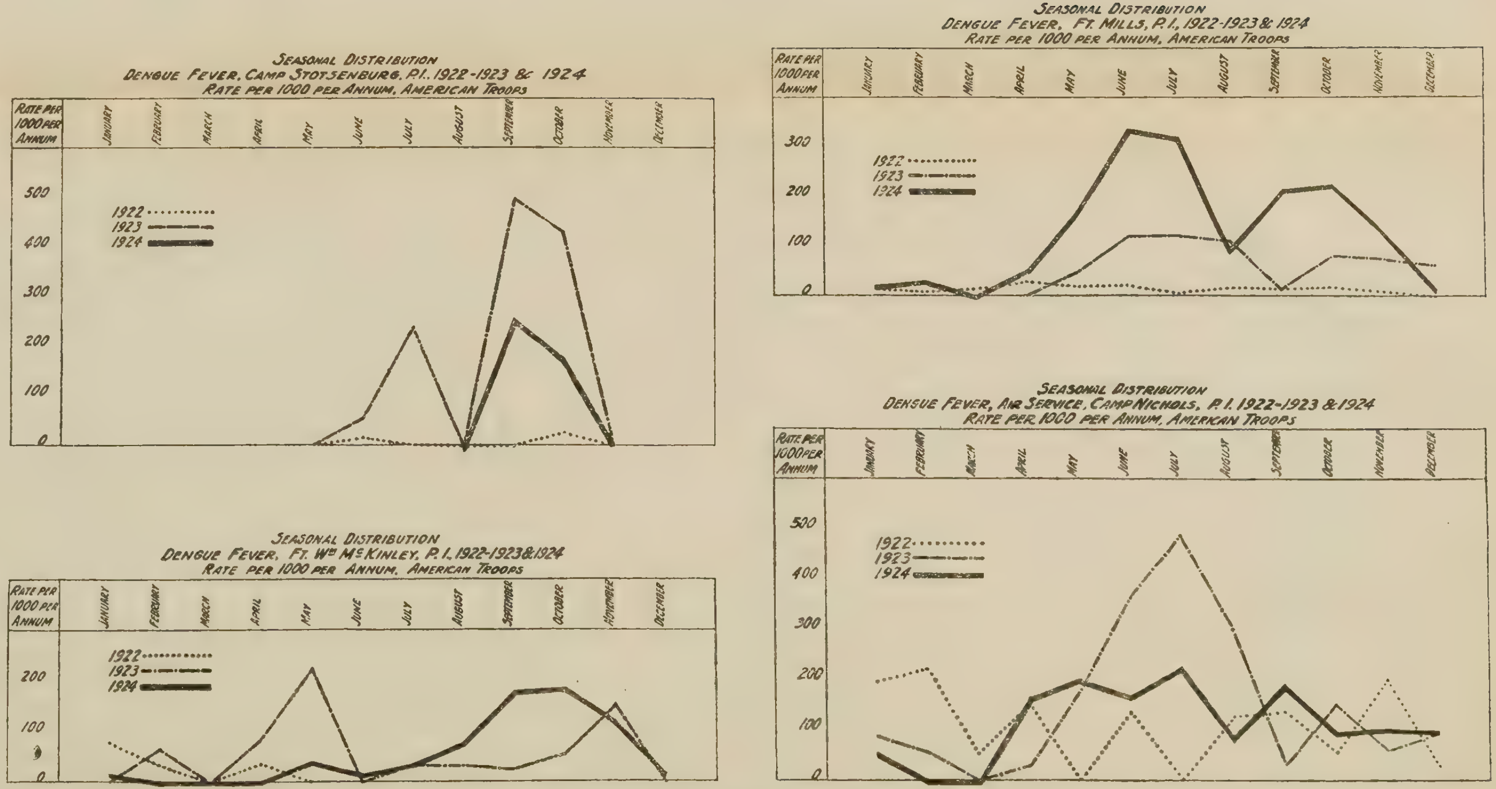
Camp Stotsenburg has been singularly free from dengue during the past three years, only three small outbreaks having occurred (two in 1923—July, August, and September—and one in 1924—September and October). This doubtless is due to a combination of two factors; namely, greater attention to mosquito control because of the location of the camp, in an area of malaria endemicity, and its great distance from Manila, which precludes constant exposure of its personnel to dengue through frequent visits to the City of Manila.



SEASONAL DISTRIBUTION
DENGUE FEVER 31ST U.S. INFANTRY, MANILA, P.I. 1922-1923 & 1924
RATE PER 1000 PER ANNUM, AMERICAN TROOPS



SEASONAL DISTRIBUTION
DENGUE FEVER, MED. DEPT. DETACHMENT, STERNBERG GENERAL HOSPITAL, P.I. 1922-1923 & 1924
RATE PER 1000 PER ANNUM, AMERICAN TROOPS



SEASONAL DISTRIBUTION
DENGUE FEVER, CAMP STOTSENBURG, P.I. 1922-1923 & 1924
RATE PER 1000 PER ANNUM, AMERICAN TROOPS

SEASONAL DISTRIBUTION
DENGUE FEVER, FT. MILLS, P.I., 1922-1923 & 1924
RATE PER 1000 PER ANNUM, AMERICAN TROOPS

SEASONAL DISTRIBUTION
DENGUE FEVER, FT. WM MCKINLEY, P.I. 1922-1923 & 1924
RATE PER 1000 PER ANNUM, AMERICAN TROOPS

SEASONAL DISTRIBUTION
DENGUE FEVER, AIR SERVICE, CAMP NICHOLS, P.I. 1922-1923 & 1924
RATE PER 1000 PER ANNUM, AMERICAN TROOPS

FIG. 9. Chart showing dengue curves at six military stations in the Philippines, 1922 to 1924. American troops only. Rate per 1,000 per annum. Same scale used in plotting rates on all charts.

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TABLE 14.—*Admissions for dengue fever, 1922, 1923, and 1924, Thirty-first Infantry (American troops).*

Year and month.	Strength.	Dengue cases, absolute numbers.	Rate per 1,000 per annum.
1922			
January	1,033	12	39
February	996	9	108
March	1,021	6	71
April	1,199	0	0
May	1,203	6	60
June	1,179	16	163
July	1,133	12	127
August	1,093	19	209
September	1,084	97	1,074
October	1,036	136	1,575
November	1,077	28	312
December	1,071	26	291
Total	13,125	367	
Average	1,094		336
1923			
January	1,076	18	201
February	1,074	19	212
March	1,118	24	257
April	1,096	51	558
May	1,070	70	785
June	1,108	62	671
July	1,025	58	679
August	995	41	494
September	1,142	13	137
October	1,153	14	146
November	1,130	10	106
December	1,097	10	109
Total	13,084	390	
Average	1,090		358
1924			
January	1,106	5	54
February	1,090	1	11
March	1,036	17	197
April	1,027	52	608
May	1,012	52	617
June	1,159	47	531
July	1,147	47	492
August	1,150	22	230
September	1,146	40	419
October	1,132	41	435
November	1,113	12	129
December	1,088	2	22
Total	13,206	338	
Average	1,101		307

TABLE 15.—*Admissions for dengue fever, 1922, 1923, and 1924, Sternberg General Hospital (American troops).*

Year and month.	Strength.	Dengue cases, absolute numbers.	Rate per 1,000 per annum.
1922			
January	139	3	259
February	131	1	92
March	130	3	277
April	148	0	0
May	135	0	0
June	158	1	75
July	159	0	0
August	160	6	450
September	158	4	304
October	133	4	361
November	144	12	1,000
December	136	2	176
Total	1,731	36	--------
Average	144	----------	250
1923			
January	118	2	203
February	115	3	313
March	141	4	340
April	152	4	316
May	141	11	936
June	193	13	808
July	132	12	1,091
August	132	9	818
September	159	4	302
October	166	1	72
November	162	4	296
December	157	4	242
Total	1,768	71	--------
Average	147	----------	483
1924			
January	164	2	146
February	157	0	0
March	152	1	79
April	132	3	273
May	133	5	451
June	158	8	604
July	175	15	1,030
August	174	10	690
September	201	25	1,493
October	200	13	780
November	206	4	233
December	194	[a] 5	309
Total	2,047	91	---------
Average	171	----------	532

[a] Includes three cases of experimental dengue.

TABLE 16.—*Admissions for dengue fever, 1922, 1923, and 1924, Fort William McKinley.*

Year and month.	American troops.			Filipino troops.		
	Strength.	Dengue cases, absolute numbers.	Rate per 1,000 per annum.	Strength.	Dengue cases, absolute numbers.	Rate per 1,000 per annum.
1922						
January	455	3	79	3,878	0	0
February	369	1	33	3,718	4	13
March	362	0	0	3,742	0	0
April	358	1	34	3,748	0	0
May	332	0	0	3,783	0	0
June	230	0	0	3,578	1	3
July	241	0	0	3,407	0	0
August	198	0	0	3,147	0	0
September	120	0	0	2,596	2	9
October	93	0	0	2,767	1	4
November	104	0	0	2,768	1	4
December	98	0	0	2,772	0	0
Total	2,960	5			9	
Average	247		20			
1923						
January	91	0	0	2,774	1	4
February	364	2	66	2,638	0	0
March	399	0	0	2,689	0	0
April	418	3	86	2,771	0	0
May	265	5	227	2,807	0	0
June	317	0	0	2,971	0	0
July	732	2	33	3,063	34	132
August	756	2	32	3,058	20	78
September	916	2	26	3,108	6	23
October	872	4	55	3,189	4	15
November	782	10	153	2,118	4	23
December	697	0	0	2,363	2	10
Total	6,609	30			72	
Average	551		55			
1924						
January	745	1	16	3,017	2	11
February	1,488	0	0	4,631	12	4
March	764	0	0	3,456	0	0
April	847	0	0	3,540	0	0
May	611	2	39	3,462	0	0
June	785	1	15	3,721	1	4
July	698	2	34	3,754	1	4
August	778	5	77	3,866	1	4
September	879	13	178	3,531	10	45
October	879	59	184	3,617	13	59
November	875	9	120	2,612	33	148
December	861	1	14	2,480	3	14
Total	10,210	93			76	
Average	851		109			

TABLE 17.—*Admissions for dengue fever, 1922, 1923, and 1924, Fort Mills.*

Year and month.	American troops.			Filipino troops.		
	Strength.	Dengue cases, absolute numbers.	Rate per 1,000 per annum.	Strength.	Dengue cases, absolute numbers.	Rate per 1,000 per annum.
1922						
January	3,491	4	14	606	0	0
February	3,020	2	8	844	0	0
March	2,914	3	12	786	1	15
April	2,708	6	27	784	0	0
May	2,667	4	18	806	2	30
June	2,527	4	19	607	0	0
July	2,388	1	5	632	1	19
August	2,292	3	16	635	0	0
September	1,901	2	13	1,018	0	0
October	1,534	2	16	1,264	0	0
November	1,429	1	8	1,435	0	0
December	1,350	0	0	1,534	0	0
Total	28,221	32			4	
Average	2,352		13			
1923						
January	1,273	0	0	1,602	0	0
February	1,167	0	0	1,659	0	0
March	1,144	0	0	1,673	0	0
April	1,023	0	0	1,674	0	0
May	1,007	4	48	1,682	0	0
June	1,080	10	120	1,691	8	57
July	787	8	121	1,602	25	187
August	846	8	113	1,586	5	38
September	843	1	14	1,628	0	0
October	1,176	8	81	1,632	4	29
November	1,163	7	73	1,631	3	22
December	1,170	6	62	1,233	0	0
Total	12,679	52			45	
Average	1,057		48			
1924						
January	1,270	2	19	1,634	0	0
February	1,258	3	29	1,638	2	15
March	1,225	0	0	1,646	3	22
April	1,220	5	49	1,646	1	7
May	1,202	17	170	1,644	5	36
June	1,290	36	335	1,642	4	29
July	1,281	34	319	1,645	10	73
August	1,478	12	97	1,641	3	22
September	1,519	27	213	1,859	12	77
October	1,505	28	223	1,861	16	132
November	1,554	17	131	1,858	6	39
December	1,552	2	15	1,855	0	0
Total	16,354	183			62	
Average	1,363		134			

TABLE 18.—*Admissions for dengue fever, 1922, 1923, and 1924, Camp Stotsenburg.*

Year and month.	American troops.			Filipino troops.		
	Strength.	Dengue cases, absolute numbers.	Rate per 1,000 per annum.	Strength.	Dengue cases, absolute numbers.	Rate per 1,000 per annum.
1922						
January	940	0	0	1,665	0	0
February	868	0	0	1,709	0	0
March	932	0	0	1,762	0	0
April	876	0	0	1,749	0	0
May	789	0	0	1,789	0	0
June	802	1	15	1,703	0	0
July	778	0	0	1,562	0	0
August	827	0	0	1,427	0	0
September	839	0	0	1,371	0	0
October	510	1	23	1,450	0	0
November	297	0	0	1,428	0	0
December	278	0	0	1,524	0	0
Total	8,736	2			0	
Average	728		3			0
1923						
January	244	0	0	1,319	0	0
February	254	0	0	1,466	0	0
March	313	0	0	1,671	0	0
April	343	0	0	1,706	0	0
May	340	0	0	1,942	3	19
June	214	1	56	1,874	1	6
July	201	4	240	1,819	6	40
August	188	0	0	1,912	13	81
September	192	8	500	1,885	7	45
October	196	7	430	1,811	2	13
November	167	0	0	1,850	1	7
December	207	0	0	1,839	3	20
Total	2,859	20			36	
Average	238		80			
1924						
January	173	0	0	1,859	0	0
February	188	0	0	1,592	0	0
March	186	0	0	1,917	0	0
April	186	0	0	1,947	0	0
May	188	0	0	1,944	0	0
June	182	0	0	1,924	0	0
July	185	0	0	1,946	0	0
August	174	0	0	1,942	2	12
September	189	4	254	1,944	2	12
October	206	3	175	1,939	0	0
November	340	0	0	1,940	0	0
December	345	0	0	1,935	0	0
Total	2,542	7			4	
Average	212		33			

TABLE 19.—*Admissions for dengue fever, 1922, 1923, and 1924, Camp Nichols (American troops).*

Year and month.	Strength.	Dengue cases, absolute numbers.	Rate per 1,000 per annum.
1922			
January	186	3	194
February	164	3	220
March	231	1	52
April	240	3	150
May	241	0	0
June	264	3	136
July	271	0	0
August	282	3	128
September	436	5	138
October	407	2	59
November	418	7	201
December	414	1	29
Total	3,554	31	
Average	296		105
1923			
January	412	3	87
February	412	2	58
March	416	0	0
April	421	1	29
May	405	6	178
June	396	12	364
July	392	16	490
August	392	10	306
September	362	1	33
October	392	5	153
November	390	2	62
December	390	3	92
Total	4,780	61	
Average	398		153
1924			
January	464	2	52
February	418	0	0
March	410	0	0
April	441	6	163
May	422	7	199
June	433	6	166
July	423	8	227
August	405	3	89
September	500	8	192
October	496	4	97
November	468	4	103
December	479	4	100
Total	5,359	52	
Average	447		116

The morbidity curve (fig. 9) for American troops stationed in Manila (Thirty-first United States Infantry and Sternberg General Hospital) shows a very different picture. The rates in those organizations are extremely high, particularly in the Medical Department Detachment on duty at Sternberg General Hospital. The relatively higher rates at Sternberg General Hospital, in our opinion, are due principally to two factors; namely, the fact that all cases of dengue among the army personnel in Manila are admitted to this hospital, thus creating a highly concentrated reservoir for the transfer of the virus to *Aëdes* mosquitoes, and the fact that during the past no effective measures have been instituted for preventing access of the *Aëdes* to patients with dengue—patients with dengue in its early stages (first three days) were not required to remain under mosquito nets, there were innumerable defects in the window and door screens of the dengue ward, no screened vestibules were provided, and the ward toilet was inadequately screened. These defects have been remedied by the conversion of the experimental ward used by this board in its transmission experiments into a receiving ward for undetermined fevers, and it is the intention of the commanding officer to hold all patients with dengue in this ward for a period of three days or more subsequent to the onset of symptoms.

At Camp Nichols, on the outskirts of Manila, the rates are somewhat low, doubtless due to more-efficient application of mosquito-control measures than is possible in Manila.

No general morbidity rates for dengue in the civil population of the Philippines are available, but this study of its prevalence in military commands suggests that it occurs and ordinarily is confined very largely to Manila and possibly to other large cities in the Islands.

In Manila many of the buildings are of a permanent type of construction with the inevitable roof gutter that becomes clogged or has depressions here and there that will hold collections of rain water. The roof gutters, together with collections of tin cans, broken bottles, etc., on the premises, and standing water in containers inside houses result in the breeding of immense numbers of *Aëdes aegypti* mosquitoes. Given a large *Aëdes* population, only two other factors are necessary to bring about epidemics of dengue; namely, cases of dengue and a large number of nonimmunes. The cases of dengue are ever present and, as Manila is a large seaport city, nonimmunes are constantly arriving in large numbers.

In the provinces, however, conditions are somewhat different. The great majority of the buildings are constructed of nipa or bamboo with thatched roofs and without roof gutters, the population is more stable, there are comparatively few nonimmunes (Americans and Europeans), and it is probable that *Aëdes aegypti* is constantly present in considerably smaller numbers than is the case in Manila.

That epidemics of dengue will and do occur at outlying military stations in the Philippines if there is the slightest relaxation in the control of *Aëdes* breeding has been demonstrated a number of times. One of the most important of such outbreaks was that occurring at Fort William McKinley in 1906, and reported by Ashburn and Craig (1907).

Figure 9 shows also that during the past three years small outbreaks of dengue have occurred at each of the outlying stations. However, the point of special interest to be brought out from the comparison of differing monthly incidence in the same years at different stations lies in its indication that strictly local conditions are vitally important in the transmission of dengue. This follows from the relative domesticity of the vector which probably seldom leaves the immediate neighborhood of its emergence from the pupal stage, but promptly seeks some house as a shelter and remains there in most cases throughout life. Ordinary observation in Manila shows that dengue is a neighborhood disease. One year one group of houses will be attacked, but may remain entirely or relatively free the next year, even with a change of occupants. These facts show that a high dengue rate is chargeable to lack of precautions in the immediate neighborhood and point very definitely to the localization of responsibility. At the same time, from the standpoint of the action of civil health agencies, they indicate great difficulty in effective control work, as the latter obviously requires the coöperation of the individual householder, a notoriously difficult thing to secure even when the disease being combatted is a fatal one, and doubly difficult in dengue.

SEASONAL OCCURRENCE OF DENGUE IN THE PHILIPPINES

There is a definite dengue season in Manila and the lowlands of Luzon, despite the facts that the atmospheric temperature is high throughout the year, only rarely dropping below 70° F. (21.1° C.) and never below 68° F. (see fig. 11); that *Aëdes* mosquitoes are more or less active throughout the year; that sporadic cases of dengue occur throughout the year; and that large numbers of nonimmunes are added to the population without interruption.

Morbidity statistics showing its occurrence at military stations by months for the years 1922, 1923, and 1924 are incorporated in Table 20 and plotted graphically in fig. 10.

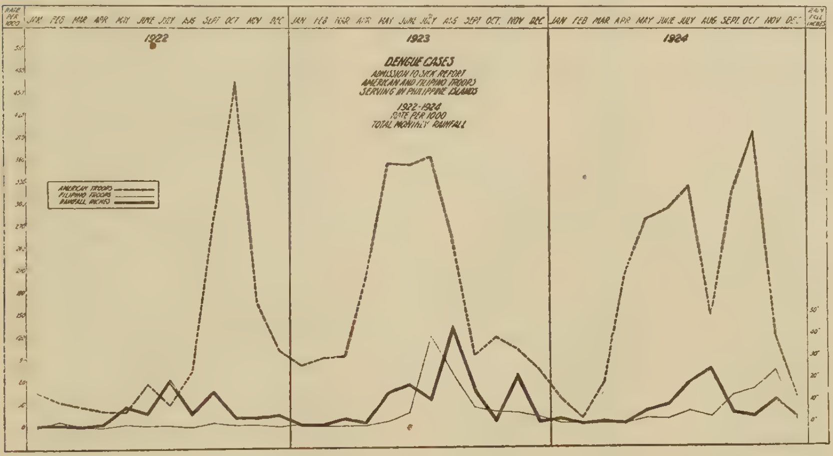

FIG. 10. Chart showing dengue cases, admission to sick report, American and Filipino troops serving in the Philippine Islands, 1922 to 1924. Rate per 1,000. Total monthly rainfall.

Inspection of the three-year monthly morbidity curve in fig. 10 shows that, generally speaking, the dengue season in Manila extends from April to November, though there is considerable variation in the curve from year to year within these limits.

In 1922 the dengue season was somewhat delayed. Noteworthy increases in morbidity did not occur until June, and the peak of the epidemic was not reached until October.

In 1923 and 1924 the season began in April and ended in November, the peak having been reached in 1923 during the month of July and in 1924 in October.

As dengue is endemic in Manila and new increments of nonimmunes are constantly being added to the population of that city, it was of interest to make some inquiry into the factor or factors that might have brought about the delay in its seasonal occurrence in 1922.

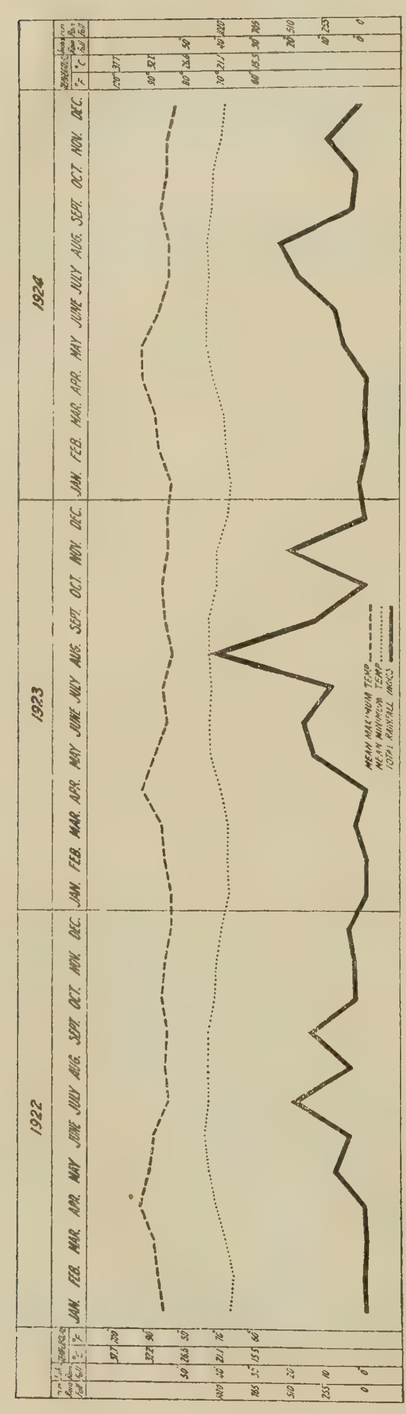

FIG. 11. Chart showing meteorological data for the city of Manila. Air temperatures and rainfall monthly, 1922 to 1924.

The most reasonable explanation was that natural environmental conditions during the early months (February to May, inclusive) of 1922 were adverse for *Aëdes* breeding, with consequent marked reduction in their numbers.

The weather reports for 1922 to 1924 were obtained from the director of the Philippine Weather Bureau and the distribution of the rainfall for the three years was plotted. The results are incorporated in fig. 11, which shows graphically the total monthly rainfall and the maximum and the minimum atmospheric temperatures in Manila, by months, for 1922, 1923, and 1924.

During each of the three years marked increases in total monthly rainfall began in May, and this might be interpreted as suggesting that the conditions for mosquito breeding were similar for the three years and that, therefore, some explanation other than rainfall must be sought for the delay in the dengue season during 1922.

We were confident that, to some degree, it was due to an enormous reduction in the *Aëdes* population occasioned by the absence

TABLE 20.—*Admissions for dengue, 1922, 1923, and 1924, from Thirty-first Infantry, Sternberg General Hospital, Fort William McKinley, Fort Mills, Camp Stotsenburg, and Camp Nichols.*

Month and year.	American troops.			Filipino troops.		
	Strength.	Cases, absolute numbers.	Cases, rate per 1,000.	Strength.	Cases, absolute numbers.	Cases, rate per 1,000.
January, 1922	6,244	25	48	6,149	0	0
January, 1923	3,214	23	86	5,695	1	2
January, 1924	3,922	12	37	6,510	2	4
February, 1922	5,548	16	35	6,271	4	8
February, 1923	3,386	26	92	5,763	0	0
February, 1924	4,599	4	10	7,861	14	21
March, 1922	5,590	13	28	6,290	1	2
March, 1923	3,531	28	95	6,033	0	0
March, 1924	3,773	18	57	7,019	3	5
April, 1922	5,529	10	22	6,280	0	0
April, 1923	3,453	59	205	6,151	0	0
April, 1924	3,853	66	206	7,133	1	2
May, 1922	5,367	10	22	6,378	2	4
May, 1923	3,228	96	357	6,431	3	6
May, 1924	3,568	83	279	7,050	5	9
June, 1922	5,160	25	58	5,888	1	2
June, 1923	3,308	98	355	6,536	9	17
June, 1924	4,008	98	294	7,287	5	8
July, 1922	4,970	13	31	5,601	1	2
July, 1923	3,269	100	367	6,484	65	120
July, 1924	3,909	106	325	7,345	11	18
August, 1922	4,852	31	76	5,209	0	0
August, 1923	3,309	70	254	6,556	38	70
August, 1924	4,159	52	150	7,449	6	10
September, 1922	4,538	108	286	4,985	2	5
September, 1923	3,614	29	96	6,621	13	24
September, 1924	4,434	117	317	7,334	24	39
October, 1922	3,713	145	468	5,481	1	2
October, 1923	3,955	39	119	6,632	10	18
October, 1924	4,418	148	401	7,417	29	47
November, 1922	3,469	48	167	5,631	1	2
November, 1923	3,794	33	104	5,599	8	17
November, 1924	4,556	46	121	6,410	39	73
December, 1922	3,347	29	104	5,830	0	0
December, 1923	3,718	23	74	5,435	5	11
December, 1924	4,519	14	37	6,270	3	6

of abundant suitable breeding places, and the rainfall statistics for 1922 to 1924 were subjected to more-detailed analysis, the results of which are shown graphically in figs. 12, 13, and 14.

These charts show the rainfall for each day throughout the three years (1922 to 1924) as recorded at the Weather Bureau Station, located centrally in the City of Manila. The argument might be advanced that, as observations are taken at only one

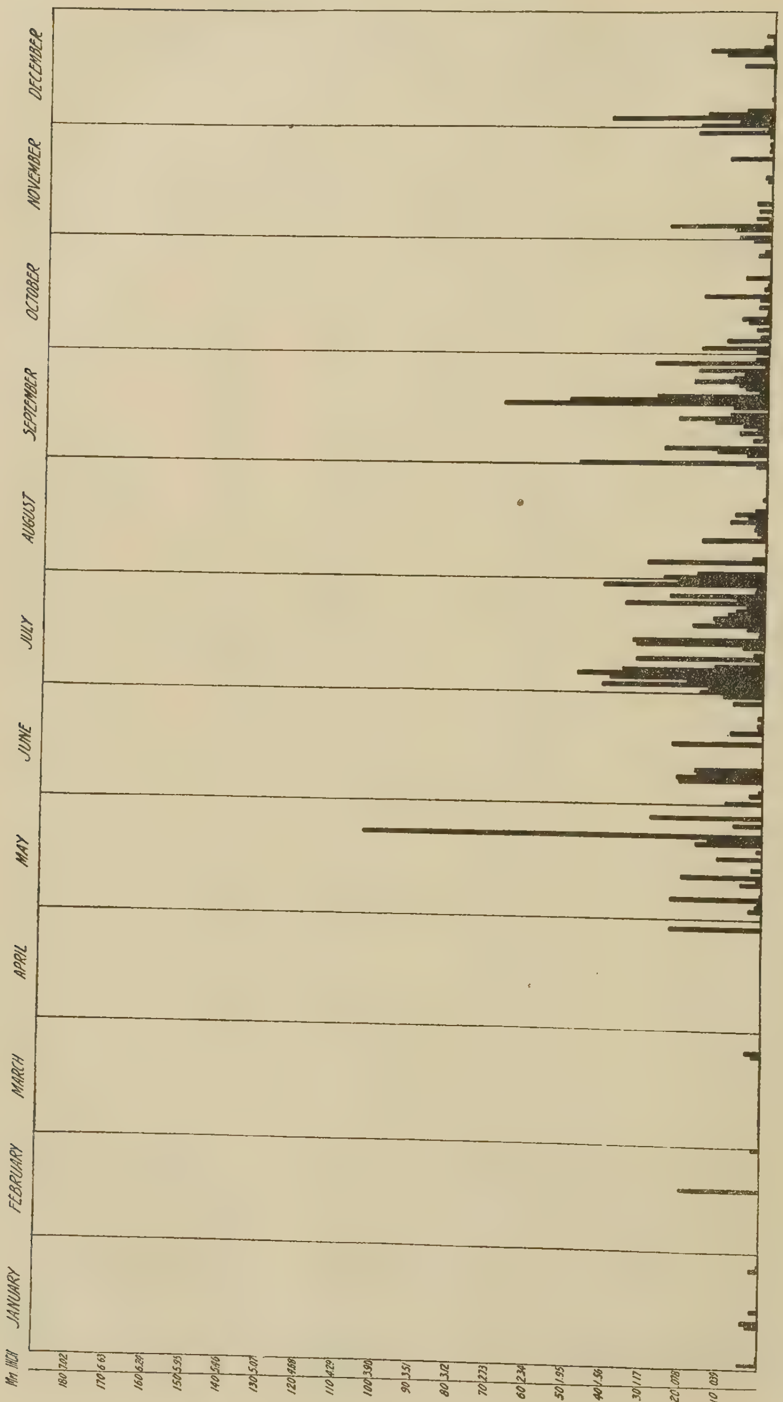

FIG. 12. Chart showing daily rainfall, Manila, P. I., 1922.

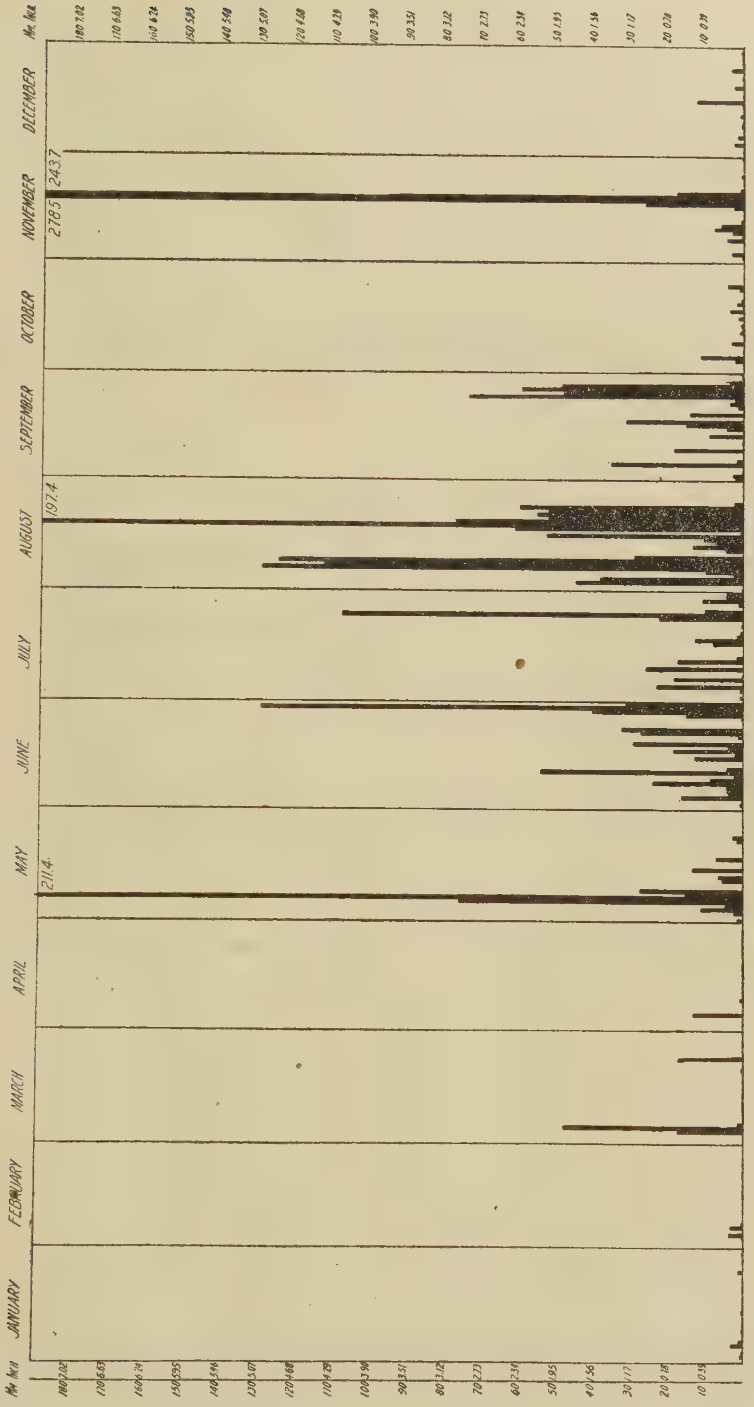

FIG. 13. Chart showing daily rainfall, Manila, P. I., 1923.

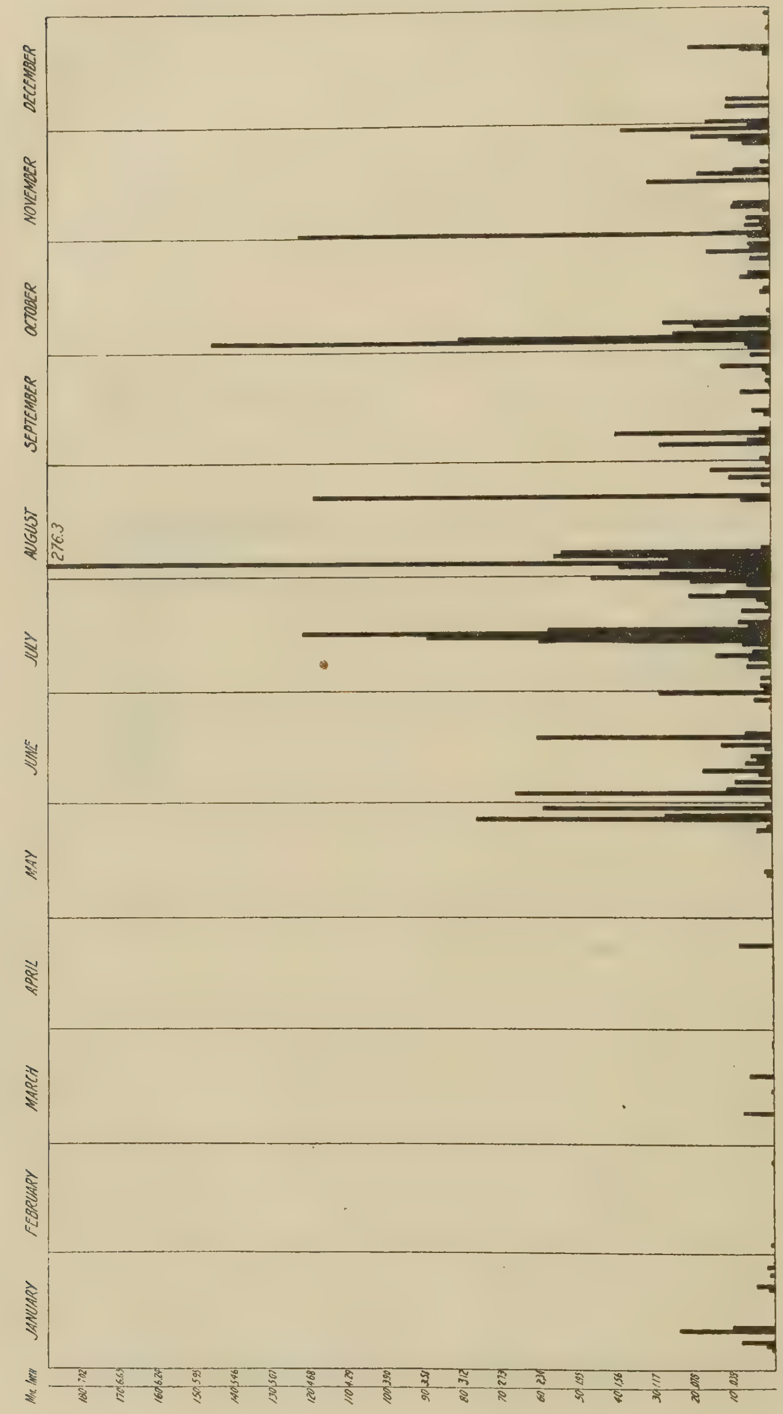

FIG. 14. Chart showing daily rainfall, Manila, P. I., 1924.

place, the figures are not reliable, as heavy showers may fall in one section of the city and other sections may have no rain whatsoever. This phenomenon is of comon occurrence at the beginning of the season of rains (June) and at the end thereof (October and November), but it occurs only infrequently during the dry season (March and April), and it can be assumed that these records reflect fairly accurately the precipitation during March and April in the City of Manila.

Before commenting on the data contained in figs. 12, 13, and 14, it is essential that we know how much rain must fall during any one day to permit water to collect in clogged roof gutters, tin cans, bottles, etc., in such quantities that even with rapid evaporation sufficient amounts will remain for from ten to fifteen days to assure a natural environment favorable for the breeding of *Aëdes.*

To determine this, we selected breeding containers comparable with those observed under natural conditions, filled them with water to graduated levels (5, 10, 15, 20, and 40 millimeters) and exposed them to varying environmental conditions (shade, sunlight, inside rooms, in the open, etc.). The observations were made in the early part of April, 1925 (the hot dry season in Manila). The following results were obtained:

Water in open Petri dishes at a level of 5 millimeters had evaporated completely in three days, usually in two, irrespective of whether exposed to direct sunlight or in the shade. Water in open Petri dishes at a level of 10 millimeters evaporated completely in from four to six days if exposed to the direct rays of the sun or left in the open air. If exposed in rooms, it evaporated completely in eight days. Water in glass jars with an initial level of 15 millimeters in one instance had dropped to 5 millimeters in five days, and in two others had completely evaporated. Evaporation was complete in all three tests on or before the eighth day. Evaporation inside rooms was complete on or before the eleventh day.

When the water level on initial exposure was 20 millimeters there was either a reduction to 2 millimeters in ten days, or complete evaporation.

These experiments suggest that if the rainfall during a single day in the dry hot season is from 15 to 20 millimeters or more, and there are no additional rains for from ten to fifteen days, a sufficient amount of residual water will remain in many artificial containers to permit the *Aëdes aegypti* to deposit eggs and these eggs to pass through the larval and pupal stages and emerge as adults. If additional showers (3 to 5 millimeters) occur every few days, breeding conditions are more favorable. If, on the contrary, the precipitation occurring in one day throughout the dry period remains at a low level (5 to 7 millimeters) there will be but little chance for *A. aegypti* to breed out in artificial containers, roof gutters excepted, even though there be repetition every six or eight days, as most of the collections of water will have evaporated (two to four days) between rains. In the case of roof gutters that have marked depressions or that are completely clogged the amount of water collected may be large, as the gutter collects the water from a large surface area.

The statement is justified, therefore, that during the dry season in Manila (March to May, inclusive), if a few heavy rains occur at intervals of from fifteen to twenty days, conditions are favorable for the multiplication of *Aëdes aegypti*, and their presence in large numbers may be expected. If, on the contrary, the rainfall during one day is small (3 to 6 millimeters), even though such rains recur every few days (five to seven days) the evaporation of water is so rapid that the *Aëdes* encounters great difficulty in maintaining itself, and it may be expected that comparatively few of these insects will be observed.

The rainfall in Manila from June to September, inclusive, is uniformly high, and environmental conditions for the breeding of mosquitoes in general are ideal. It is during these months, however, that typhoons usually and most frequently occur, the rainfall is heavy, and at times is continuous for a week or so. Typhoon weather is, in some respects, not conducive to the multiplication of mosquitoes, as the eggs and larvæ are carried away by the heavy washing rains.

It was noted by us in 1924 that there was temporarily a marked decrease in admissions to hospital for dengue after typhoon weather that extended over a period of a week or ten days.

Having discussed somewhat in detail the general characteristics of the rainfall in Manila at various seasons of the year, its seasonal distribution, and its bearing on the mosquito population at various seasons of the year, it remains to determine whether the seasonal distribution of the rains during the dry season of 1922 differed materially from that of 1923 and 1924, and whether this factor of itself might possibly serve to account for the delay in the appearance of dengue in epidemic form in 1922 as compared with the two subsequent years.

In 1922 the admissions to hospital for dengue fever did not begin to show a seasonal increase until the month of June (fig. 10). Inspection of fig. 12 indicates that, so far as rainfall is concerned, conditions for *Aëdes* breeding were unfavorable until about the middle of February (February 17) when a fairly heavy rain fell (21 millimeters). After this date, rains were not sufficiently heavy until April 28 (two and one-third months) to provide numerous breeding places for *Aëdes.* The dry weather and the reduction in breeding places doubtless brought about an enormous reduction in the *Aëdes* population, and it is probable that not until the latter part of May—during which month there were frequently recurring rains—had breeding so increased as to produce a large *Aëdes* population.

In 1923 and 1924 the admissions to hospital for dengue increased very markedly during April (fig. 10), two months in advance of the 1922 dengue season.

It is evident from figs. 13 and 14 that the distribution of the rainfall during the early months of 1923 and 1924 differed materially from that observed during the corresponding months of 1922. In January and February, 1923, there were several rainy days, but the rainfall was too meager to assure the provision of breeding places of *Aëdes.* In the early days of March there was a heavy downpour for two days and a light rain on the following day. These heavy rains doubtless left in their wake numerous ideal breeding places for *Aëdes.* After two weeks of dry weather another fairly heavy rain fell (17 millimeters) and this was followed in twelve days (April 5) by a third day of rain (13 millimeters).

The three rains during March and April, 1923, were each sufficiently copious and occurred at sufficiently short intervals to assure large progressive increases in the prevalence of *Aëdes;* the assumption is therefore justified that the distribution of the rainfall during the dry season of 1923 can be correlated with the increase in morbidity rates for dengue during April, 1923.

During January, 1924 (fig. 14), the rainfall was sufficient in volume and occurred at such intervals as to provide an ideal environment for the breeding of *Aëdes.* During February there were only two rainy days (light showers) and breeding conditions were unfavorable. On March 9, 8 millimeters of rain fell, and on March 19, 6 millimeters. These two rains, supplemented by showers at intervals of a few days, doubtless provided some breeding places for *Aëdes.* The next succeeding rain of any consequence occurred about a month later. Breeding conditions during the dry season of 1924 were not so favorable as during the corresponding months of 1923, but were more so than during the corresponding period of 1922.

The increment of nonimmune military personnel arriving during each of the three years was approximately the same.

The tentative conclusions drawn from this inquiry, which require further confirmation, are that a study of the amount and distribution of the daily rainfall in a given area will enable one to predict increases or decreases in the *Aëdes* population, and that these factors will influence the dengue morbidity rates.

Public-health authorities are coming more and more to realize that the fuller the knowledge of the life habits of mosquitoes and of the physical conditions under which they multiply the more readily and the more intelligently can they be attacked at their most vulnerable points; that much of the time, energy, and money expended in the past on mosquito-control projects might have been conserved had our knowledge of these insects been greater; and that, with the limited funds usually available, efficiency demands that control measures be limited to those of greatest proven value.

With regard to the eradication of *Aëdes aegypti* in the Philippines, the observations made by us suggest that during somewhat prolonged dry periods, with but little rainfall, the breeding of *Aëdes* in roof gutters, cans, bottles, and other rain-water receptacles can be disregarded and that concerted efforts should be directed against their breeding places in houses (vases and other flower containers, drip cans, fire buckets, water barrels, etc.). Throughout the greater part of the year, however, when the rainfall is heavy, all types of breeding places must be attacked.

SUMMARY

Dengue has no mortality and is not a reportable disease in the Philippine Islands. It but infrequently attacks the native population in recognizable form, and it is assumed that this is due to an acquired immunity. A study of its epidemiology in the Philippines, therefore, is limited to a consideration of its occurrence in the Caucasian residents, particularly those recently arrived. As morbidity statistics relating to its occurrence are obtainable only from army sources, this discussion is based on its occurrence among army personnel.

Analysis of the morbidity and mortality statistics for all diseases in troops during the past twenty-one years indicates that great progress has been made in the control of transmissible diseases. The decline has been of such magnitude that the sickness and death rates for the latter half of the period approximate only half of those recorded for the first half. No such progress in prevention can be reported for dengue, and three of the worst epidemics ever experienced by American troops serving here were those that occurred in 1920, 1923, and 1924. During the twenty-one years, the average number of days lost from duty by military personnel because of dengue approximated seven thousand days a year.

It was found that venereal diseases, malaria, and dengue were the principal causes of sickness. Material progress has been made in the prevention of venereal diseases and malaria, but none can be reported for dengue.

A study was made of the occurrence of dengue during the past three years at six military stations, and it is shown that the disease attacks continuously and with greatest frequency troops stationed in Manila. It is further demonstrated that epidemics of dengue but infrequently occur at outlying military stations (Fort William McKinley, Camp Stotsenburg, and Fort Mills). The factors responsible for inequalities in its geographical distribution are discussed.

The dengue season in Manila extends from April to November, though there is considerable variation within these limits. In 1922 the season did not begin until June, whereas in 1923 and 1924 it began in April.

The most reasonable explanation of the delay in 1922 is that there must have been a marked reduction in the *Aëdes aegypti* population during the early months of that year resulting from adverse environmental conditions. To test the validity of this assumption a study was made of the daily rainfall during the three years. It was found that less rain fell during the early months of 1922 than during the same months in 1923 and 1924, and that environmental conditions undoubtedly were less favorable for the breeding of *Aëdes* in 1922 than was the case during the same season for the two succeeding years. As the two other factors necessary for the occurrence of dengue in epidemic proportions were present—cases of dengue and a large number of nonimmunes—the conclusion is drawn that the factor responsible for the delay in the epidemic in 1922 was a reduction in the *Aëdes aegypti* population.

SPECIFIC ETIOLOGY OF DENGUE

Although one member of the board had devoted the major portion of his time to the subject of the specific etiology of dengue for the year preceding the initiation of the transmission experiments, it should be stated at the outset that nothing of positive value has been accomplished. Certain negative findings deserve record, however, as bearing on the interpretation of results attained by others and as guides to further work along this and related lines. Here also should be emphasized the relation that the transmission results bear to the study of the virus itself.

It has been repeatedly shown by others (Ashburn and Craig, 1907; Cleland, Bradley, and McDonald, 1918, 1919; Chandler and Rice, 1923; Koizumi, Yamaguchi, and Tonomura, 1917) that the virus of dengue is present in the circulating blood of the patient and that the disease may be directly transmitted from patient to patient by injection of the infected blood. So far as can be judged from the records, the incubation period of cases thus infected does not differ materially from that observed in those infected through the bites of mosquitoes, except when inoculation is made by the intravenous route. The virus has been demonstrated by human inoculation in the whole blood, the separated serum, and in the washed corpuscles (Cleland, Bradley, and McDonald, 1917). It has been shown that filtration of infected blood or components of blood through filters shown to retain ordinary bacteria does not remove the dengue virus in all cases (Ashburn and Craig, 1907), though negative results occur in a certain proportion of trials. These negative attempts at filtration are explained by Cleland, Bradley, and McDonald (1919) as possibly due to partial clogging of the filter pores by formed elements of the blood and consequent greatly increased difficulty in penetration. The stage of the disease at which the blood is infective is of great practical interest to one who contemplates attempts at isolation of an organism by cultural methods. Transmission by means of mosquitoes has been shown by the results of the present work to be most certain if the infective biting takes place on the first day of the disease or shortly before. Positive results have been observed in one-third of the third-day bitings, but none later than that. We have made no attempt to determine how late in the disease the blood may prove infective, but it seems probable a priori that, on account of the relatively large amount used, the infectivity of the transmitted blood may persist somewhat longer than the period during which it is possible to infect mosquitoes. Cleland and Bradley (1918) demonstrated the presence of the virus on the second and third days of the disease, and they consider that two of their experiments may possibly be interpreted as indicating its presence as late as the eighth day. It appears evident, however, from the observations recorded at this time and by others, and from the analogy with similar observations in the case of yellow fever, that, to obtain positive results with certainty, the blood should be drawn from the patient as early in the disease as possible, and that blood drawn much later than the third day after onset can hardly be expected to give positive results. During these early days, however, the virus appears to be present in very small amounts of the blood. Its transmission by mosquitoes indicates that in a large proportion of instances the small fraction of a cubic centimeter removed by a single insect contains infective material. Infection by the bites of single mosquitoes are recorded (Graham, 1903; Armstrong, 1923). In the series of transmission experiments here recorded, no instance of infection from the bite of one mosquito occurred, though in two cases dengue resulted after but two mosquitoes had bitten the volunteer. No special attempt was made, however, to induce the disease by single bites, as the experimental material was naturally limited, and other matters were considered more important. Koizumi, Yamaguchi, and Tonomura (1917) report successful transmission of dengue by the injection of 0.00005 cubic centimeter of blood.

From the standpoint of the seeker for the virus of dengue, it thus appears that the most important fact brought out by these observations is that the blood to be investigated should be obtained from the patient on the first day of the disease. While it may prove possible to isolate an organism later when proper methods are developed it seems certain that the highest proportion of successful results will be attained from first-day blood. As an aid to the interpretation of results it seems also certain from the transmission experiments quoted that results obtained with blood drawn much later than the third day of the disease should not be expected to contain the virus. The possibility must be considered that the virus may exist in the blood during these later days in a form or phase of its existence in which it is not capable of exciting the disease in man, but that it might be capable of doing so after passage through the mosquito, after the manner of the gamete forms of malaria. The negative results of later mosquito bitings, however, serve to render this hypothesis extremely improbable.

The direct examination of dengue blood in stained smears and by means of the dark-field microscope has been undertaken by a number of investigators. Graham (1903) described what he believed to be an intracellular hæmatozoön, and the cause of the disease. Nagib Ardate (1910) believed that he saw the same parasite, but numerous other students of dengue blood have failed to confirm the observation and it is now generally regarded to have been due to some misinterpretation of the appearances observed. The same criticism appears to attach to the observations of Eberle (1904), Reiche (1906), and Holt (1923). Couvy (1914, 1920) reported the finding of short slender spirochætes with few turns and pointed ends in cases which he believed to be dengue. There were only a few, and they occurred shortly before onset and during the first two days of the disease. Blood containing these spirochætes produced a corresponding disease in rabbits, and the spirals were demonstrated in their blood as well. However, in as much as Couvy believes that he transmitted the disease in question by means of the phlebotomus fly, and the clinical description might apply equally well to pappataci fever, it seems probable that his results do not apply to dengue.

The analogy, epidemiologically, and to some extent clinically, between yellow fever and dengue has led to the suggestion by Craig and others that the virus of dengue when isolated will prove to be similar to that of the former disease. When Noguchi, in 1919, described his *Leptospira icteroides,* this prediction was generally recalled, and subsequent attempts to cultivate the virus of dengue artificially have included attempts by methods similar to those used by Noguchi. So far, however, it has not proven possible to show the presence of a leptospira in dengue. Cleland, Bradley, and McDonald (1918) inoculated guinea pigs and other animals and failed to find leptospiræ in their organs when the animals were killed after an appropriate interval. Chandler and Rice (1923) attempted cultivation by Noguchi's methods, with negative results, as also did Harris and Duval (1924), so far as leptospira is concerned.

Many attempts have been made to find an experimental animal susceptible to dengue. If such could be found it would greatly simplify the problem facing the student of the specific etiology of the disease. With few exceptions, however, investigators have reported negative results in their attempts to induce dengue in lower animals. The recently reported work of Duval and Harris (1924) is the most definite claim of animal transmission, and deserves on that account special discussion. Koizumi and his coworkers in Formosa (1917) believed that they had transmitted the disease to guinea pigs through two passages, though subsequent attempts failed. They failed also with dogs, white mice, rabbits, and monkeys. Cleland (1918) failed with rabbits and guinea pigs. In one instance he injected the blood of an experimental guinea pig into a human volunteer eight days after the pig had been inoculated with known infective blood. No signs of distress developed. In the course of the present work a similar attempt was made with hogs with negative results. Chandler and Rice (1923), Lavinder and Francis (1914), and Armstrong (1923) report failure to infect animals. In view of all these negative results the findings of Duval and Harris (1924) must be reviewed critically. It is well known to those who have followed temperature records and leucocytes of rabbits and guinea pigs, especially of the latter, that great variations in temperature and in blood count occur independently of disease. This fact renders it imperative to place experimental animals under observation for some days before inoculation and should make one extremely conservative in the interpretation of results. In the course of this work and also in similar observations during the study of the virus of influenza we often observed spontaneous variations in the temperature and blood count of control animals which could easily have been interpreted as disease reactions had they occurred subsequent to inoculation. Such results as those shown in the article of Harris and Duval (1924, text figure 1) are not often seen, however, and in the absence of any other cause of febrile reaction would be extremely convincing if regularly observed. It is to be noted, however, that these workers record the presence of infection by bacteria of the paratyphoid group among their experimental animals and this renders their temperature records still more difficult of interpretation. Further study of their case records shows that two of their successful experiments were made with blood obtained on the fourth day of the disease, one on the tenth, and one (case 6) on the seventh. (Case 6 is entered in their table as of the third day of the disease, but the protocol dates show the onset seven days previously.) The considerations given above with regard to the improbability of obtaining infective material late in the course of the disease serve to arouse doubt as to the nature of the process observed in guinea pigs inoculated from these cases. Duval and Harris have also reported their belief that they have cultivated from these cases and from experimentally infected animals a minute "globoid body" morphologically much like that described for acute poliomyelitis. The observations made in the course of our work which bear upon this matter will be detailed later.

In addition to yellow fever, which has already been noted as resembling dengue epidemiologically and clinically, there exists in tropical and subtropical countries a whole series of clinically related diseases in general similar to dengue. They appear to resemble dengue clinically, though individual outbreaks may differ considerably in one or another respect, though perhaps not more than is the case in different outbreaks of accepted dengue. The question as to the unity or diversity of these diseases, whether or not they should all be regarded as dengue, is discussed in "Clinical aspects of dengue." Here, however, it is important to mention one series of cases reported by Dutch investigators in Sumatra under the name *Spirochaetosis febrilis.* This disease has been studied by Vervoort (1922), Van der Velt (1923), and Kouwenaar (1924). The outstanding fact in their work is that they have succeeded in cultivating from a large proportion of clinically similar cases a spiral organism which they describe as morphologically like the organism of infectious jaundice and the leptospira of Noguchi. They have proposed for it the name *Leptospira pyrogenes,* though they admit that they have no means of definitely separating it from the leptospira of infectious jaundice. The disease with which this organism is associated is a short-lived febrile condition, without fatality, having a temperature course indistinguishable from that of dengue. In several ways, however, it appears to differ from the latter disease. Primarily, it would appear from their records that the disease is practically limited to the native races and that it spares Europeans. This, of course, is not the case in dengue as seen in the Philippines. Secondly, rash, so nearly constant in dengue, is seldom observed in their disease, never in characteristic form. Thirdly, the rather frequent occurrence of jaundice in the cases they describe seems to relate them rather to Weil's disease than to dengue, in which jaundice is infrequent. It is never seen in the Manila cases. Doctor Vervoort writes:[11]

> The question whether the fever, caused by leptospiræ, has something to do with dengue fever, is still open, as we never see a real epidemic of dengue in the typical way. It is a matter of fact that some cases show jaundice. I do not know whether you meet jaundice amongst your dengue fever cases. I must confess that there are a lot of fever cases, that have the same clinical aspect, where no spirochætes are found, but on the other hand I have never met with leptospiræ in fever cases that were not more or less typical.

This association of leptospiræ with a condition clinically similar to dengue, even if the disease is eventually recognized as different, renders it the more important to exhaust every method of finding a similar organism in dengue before it can be stated that a leptospira is not concerned in the disease. The technic used by Vervoort in his successful cultivation of this organism will be discussed later.

EXPERIMENTAL WORK

Direct examination of blood.—In view of the reported finding by Blanchard and LeFrou (1923) of spirochætes in blackwater fever by means of triple centrifugation of the blood, the first step in the hunt for the organism in the present investigation consisted in the application of their method to dengue blood. This involves the rapid separation of the red cells by a preliminary slow centrifugation, followed by the separation of the leucocytes from the supernatant fluid by more-prolonged treatment, and finally the rapid and complete centrifugation of the nearly clear fluid. This final sediment is composed mainly of blood platelets. In practice it was found that if the first two steps were combined the final results were the same and time was saved. The blood used in these centrifugation experiments was obtained from patients admitted to Sternberg General Hospital in whom dengue had occurred naturally. The active coöperation of the hospital authorities permitted the board to be notified at once on the admission of cases of dengue or even of suspicious cases. Many of these, admitted as "suspect dengue" or "fever, cause undetermined," proved later to be other than dengue and served as excellent control material.

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[11] Personal communication.

The examination of the final sediment from the blood of these cases always showed varying numbers of actively motile wavy filaments having the appearance of living organisms, but quite different in appearance under the dark-field microscope from the leptospiræ. The application to dried films of this material, of the Giemsa stain, and of the silver-agar-silver impregnation method of Warthin and Starry resulted in the demonstration of spiral filaments in many cases indistinguishable from those seen in cultures of leptospiræ, or in preparations of spiral organisms commonly found in the mouth. It was noted, however, that both under the dark field and in stained preparations there was a lack of uniformity in the various individual filaments observed, sufficient to make it seem improbable that they could all be individuals of the same species. Accordingly, similar preparations were made from known normal blood and it at once developed that the forms seen were in no way peculiar to dengue. That these pseudo-spirochætes had been repeatedly recorded in the literature was discovered on investigation of the subject. Indeed, they have repeatedly been reported as the causative organism of various diseases. A more-detailed description of these confusing filaments and a suggested means by which they can be distinguished from true spirochætes have already been made the subject of a special report by this board. Suffice it to say here that they can readily be found in all blood samples if looked for, and are more numerous in blood from fever cases and in blood which has suffered the action of deteriorating agencies such as time, undue heat, etc. The appearances under the dark field serve to distinguish them clearly from any of the known types of spiral organism. Although this finding proved entirely negative so far as the etiology of dengue is concerned, the fact that these pseudo-spirochætes are often entirely indistinguishable from true ones in stained preparations serves to warn against depending upon stained material from the blood in a further hunt for leptospiræ in this disease.

While on the subject of pseudo-spirochætes it may be stated that these filaments are regularly seen, not only in blood preparations, but also in culture tubes containing blood from patients, if the medium used in cultivation is isotonic with the blood. In such media, the blood of the patient, on standing for some days, settles out in a layer at the bottom of the tube. The upper layer of the blood sediment, consisting very largely of leucocytes, is seen upon dark-field examination to be very rich in actively motile filaments of the type described. The knowledge of the existence of the pseudo-forms, however, served to guard against a conclusion that a spirochæte was growing in the tube. A medium, in which the added blood is laked, seems to prevent the formation of these pseudo-spirals, or to destroy them when formed. They have never been observed in nonisotonic culture media.

In view of this peculiarity of the false forms, when the work of Connor (1924) on the separation of the organism of spotted fever from the blood by means of centrifugation of diluted serum appeared, it seemed worth while to apply his method to dengue blood, as it might prove that the organism was of such specific gravity as to be thrown out by centrifugation from diluted serum though remaining in suspension in the undiluted medium. When this attempt was made there were available from time to time various cases of experimental dengue which made it possible to be certain that the material was obtained within a few hours of the actual onset of the disease. From four cases of this type, 20 cubic centimeters of blood were obtained within twenty-four hours of onset. The blood was drawn into sterile tubes containing a small quantity of citrate of sodium and was at once conveyed to the laboratory. It was then subjected to a light preliminary centrifugation to separate the red cells, and the supernatant fluid was removed to other tubes. Two parts of sterile distilled water were then added to each part of the nearly clear plasma and the diluted material centrifugated at about 3,000 revolutions for three hours. The small amount of sediment from each tube was spread on cover slips, fixed in absolute alcohol, and stained by Giemsa and by silver impregnation. So far, this method has failed to reveal the presence of spiral organisms or any other form that can definitely be regarded as a microörganism, or that differs from the appearances seen in normal blood subjected to the same treatment. In both dengue blood and controls there are frequently seen, in Giemsa preparations, minute deeply staining rounded bodies, often in pairs and frequently surrounded by a narrow clear zone resembling a capsule. These bodies are observed in all preparations from blood or its components and most probably represent particles of protein material which have taken the stain. Similar minute particles are sometimes seen in silver preparations. They are, as a rule, barely large enough to be visible with a 1.8-millimeter objective and they vary greatly in number, from enormous numbers to relative scarcity.

Cultivation experiments.—In attempts at cultivation the selection of methods has been based on the probability already discussed, that the organism of dengue will ultimately prove to be a leptospira or some closely related type. The blood used in the majority of our attempts at cultivation was obtained by the same method as was used in the earlier investigations of centrifugated blood. Prompt notification of admissions to the hospital enabled us to obtain samples of blood within a few hours, and the considerable number of cases thus cultured, which proved on further investigation to be other than dengue, served as unselected control material. The finding in cultures on several occasions of appearances suggestive of growth aroused hopes that we were about to attain positive results; however, on investigation we found that certain of the cases showing such appearances were not dengue but proved to be something totally different.

Three types of culture media have been mainly used. Two of these, the rabbit kidney ascitic fluid medium of Smith and Noguchi and the semisolid plasma medium used by the latter in his cultivation of *Leptospira icteroides* from yellow fever cases, were the same as those used by Duval and Harris in the work already referred to (1924) and are sufficiently well known to require no special description. The third medium is that used successfully by Vervoort in his cultivation of leptospiræ from his spirochætosis cases in Sumatra (1922). This, in the form now used by Vervoort, consists merely in a 1:1000 solution of peptone, to which is added normal "acidum phosphoricum" in the proportion of 0, 1, 2, and 3 drops to 100 cubic centimeters. The blood from the patient is taken without citration, allowed to clot, and is then mixed as much as possible so that the inoculum contains both serum and cells. He uses "one or more" drops of this serum to each tube of the medium. He seldom finds the leptospiræ "earlier than a fortnight after the inoculation and very often still later." Most of the work done by us was based on his earlier description of his medium which had a p_H value of about 6.6. In each experiment from six to ten tubes were inoculated. In as much as inoculated tubes had to be incubated over prolonged periods it was found advisable to use a loose rubber cap over the top of the tubes to reduce the probability of mold contamination which is frequent in Manila, especially in the rainy season.

The inoculated tubes were examined at intervals up to a month or more by means of the dark-field microscope and by various staining methods. In none was any evidence of leptospira found. Samples of the material in the tubes were removed from different levels by means of sterile capillary pipettes and subjected to examination, and the tubes were returned to the incubator. In about one-third of the cases the tubes were not placed in the incubator but were allowed to stand at room temperature which, during the dengue season, averages slightly above 80° F.

In the examination of material from the culture tubes under the dark field it was early observed, especially if the material had been taken from the bottom of the tubes, that enormous numbers of highly refractile dancing bodies, much smaller than ordinary cocci, were present. These bodies differed in no recognizable way from those seen in the examination of any other serous material except in the markedly greater number present as compared, for instance, with the number seen in fresh blood, or the serum from a suspected chancre. The stained preparations from such material also showed in most instances large numbers of the minute rounded bodies already described in the discussion of the results of the examination of centrifugated blood. Often these bodies were very numerous in such material and seemed sufficiently distinct and uniform in appearance to suggest that they were minute rounded microörganisms. This observation, however, also proved to be valueless, as examination of control material, both from hospital cases that proved to be other than dengue and from normal individuals, showed that identical appearances occurred when nondengue blood was used.

The gross appearance of the culture tubes proved to be of little use to us. In any given set of inoculated tubes, there was considerable variation in appearance in the course of weeks. Certain tubes would hæmolyze more rapidly than others, and in many the blood would take on a brownish appearance. Careful investigation of these developments showed no corresponding differences on examination of the tube contents under the dark field or by staining methods. Tubes of the Vervoort medium, already hæmolyzed, showed no change on standing except the separating out of a sediment of laked red cells. One appearance, however, did present itself in the case of many of the tubes of the semisolid plasma medium and for a time raised high hopes of definite results. This consisted of the gradual formation, in the semisolid portion of the tube, of definite colonylike appearances. After some weeks' standing, these sometimes attained a diameter of a millimeter or more and thus could be very readily seen with the unaided eye. Other tubes showed similar "colonies," but much smaller, and in some it was necessary to use the hand lens to distinguish the appearance at all. The most careful microscopic examination of these colonylike masses failed to show any appearance in any way different from those seen in other tubes which showed nothing of the kind. Darkfield and staining methods both failed to show anything other than the minute rounded bodies already mentioned. Finally, it was shown that the addition to the semisolid medium of known normal blood resulted in the same type of apparent colony formations after standing for several days. In no case, whether dengue blood or normal blood was used, did all the tubes inoculated show this change, and those showing it varied greatly in degree.

Cultures were made by these various methods from forty-five dengue cases, many of which were investigated by more than one of the culture methods. Only four were inoculated into the Smith-Noguchi rabbit-kidney tubes on account of the difficulty in obtaining satisfactory tubes of the medium, partly owing to uncertain supply of ascitic fluid and the great liability to mold contamination, and partly because the medium itself is not the best for the growth of the special type of organism for which we were looking. We feel that the number of cases investigated is sufficient to convince us that the blood of the cases used contained no leptospiræ cultivable by the methods indicated. The possibility that our cases were obtained too late for successful results, in spite of the precautions taken, will be indicated below.

Animal experiments.—Only a few attempts were made in the course of this work to transmit dengue to lower animals. Two attempts were made with rabbits, two with guinea pigs, and two with hogs. The results were entirely negative, but the small number of trials renders conclusions on the grounds of present work impossible.

Examination of infected mosquitoes.—During the course of the mosquito-transmission work large numbers of mosquitoes of lots known to be capable of transmitting dengue became available. These were examined in considerable numbers by Dr. Andrew W. Sellards, who was at the time engaged in research work at the Bureau of Science. His results, which are of considerable theoretical interest, may be reported by him. When it became probable that his findings had no direct relation to the etiology of dengue, a few mosquitoes, all then available, were subjected to dissection, and the viscera spread on cover slips and stained by the silver-impregnation method. In several mosquitoes thus examined small, extremely delicate, spiral filaments were observed; they occurred in groups, and where found were numerous, and were uniform in size and shape. Time has not yet permitted the repetition of this method of study, nor has a sufficient number of uninfected mosquitoes been examined to ascertain whether or not similar appearances exist in them.

DISCUSSION

With regard to the entirely negative character of the results of the work here reported, it is felt that one factor may have invalidated it to the extent that no definite negative conclusions should be drawn at this time. It will be remembered that during the course of our transmission experiments all attempts to infect mosquitoes from cases of dengue that occurred naturally proved fruitless. These attempts were made with the very earliest cases on which it was possible to establish even a presumptive diagnosis of dengue and thus were made at a stage of the disease closely corresponding to that at which blood was taken for use in our attempts at cultivation. In the section on the Clinical characteristics of dengue it is shown that the average duration of fever in our experimental cases was three and nine-tenths days, while the average duration of fever after admission to the hospital in twenty-five consecutive cases of dengue admitted to the hospital during the same period was only one and nine-tenths days. This can mean only that, on the average, cases during that time were admitted to the hospital on the third day of the disease. Taking into consideration, then, the results obtained in infecting mosquitoes on the different days of the disease, and the results recorded here and elsewhere as to the infectivity of the blood on direct transfusion, it appears evident that no negative reports of any kind should be regarded as conclusive unless based on material obtained during the first twenty-four hours of the illness. This is possible in dengue, practically speaking, only if the material is obtained from experimentally induced cases, and the further work of the board along these lines will be based on the intensive study of a relatively small number of such cases, rather than on cultivation from a larger number of cases in which it is impossible to know the exact stage of the disease.

Our negative results, however, have one point of value; that is, in regard to the interpretation of the recent work of Duval and Harris (1924). We have already pointed out the reasons why it is difficult to judge the fact of transmission of a disease of this kind to lower animals, and have indicated certain special reasons why, in this particular instance, the interpretation placed by Duval and Harris on their results in guinea pigs is open to argument. Their description of results obtained in the semisolid plasma medium with dengue blood we have been able to parallel completely, not only with blood from dengue, but also with blood known to have no relation to that disease. Unfortunately, Duval and Harris appear to have omitted a description of their results in this medium with normal blood. We feel therefore that, though their claim that they had transmitted the disease to guinea pigs is open to confirmation, the grounds on which they based their belief that they had cultivated an organism from their dengue material are extremely slight, as all the appearances observed, whether the gross changes in the inoculated tube or the microscopic findings under the dark field and with stained preparations, can readily be duplicated with nondengue material.

Our conclusion is that the specific organism of dengue remains to be discovered and that, as already indicated, the best line of attack for future work lies in the use of material from cases known to be in the first day of the disease.

CLINICAL ASPECTS OF DENGUE

INTRODUCTION

The clinical study of the experimental cases which form the subject of this report was primarily undertaken for the purpose of establishing beyond question that the disease with which we were working was in fact dengue. This demonstration is essential as a preliminary to all the conclusions drawn. The existence of a whole group of denguelike diseases in tropical and subtropical regions has led to a great deal of discussion as to whether the disease manifests itself somewhat differently in different regions and in different outbreaks, or whether there are in fact several essentially different infections of similar clinical character. In certain regions, pappataci fever and dengue may readily be confused on account of their similar character although, as will be shown later, there are sufficient points of difference clinically to distinguish epidemics, at least, even if sporadic cases may readily cause confusion. The fever of van der Scheer, the seven-day fever described by Sir Leonard Rogers (1919), and a number of similarly denominated short-lived febrile conditions may or may not be in fact dengue, and the final decision very probably awaits the demonstration of the active inciting cause. In the meantime, however, it is possible that the study of the variations occurring in a group of dengue cases known to be due to the same strain of virus may throw some light on the matter and prove valuable as well from the standpoint of diagnosis. Of the forty-eight positive mosquito transmission experiments, forty-two were due definitely to the same strain of virus; all originated from the cases infected by the blood of Private Collins, who had at the time a typical case of dengue. (See Appendix.) The other six cases were transmitted by mosquitoes infected from a case occurring in one of us (J. F. S.). This case occurred simultaneously with two of our experimental cases and was in all probability due to the escape from their cage of several infected mosquitoes on the occasion of their transfer to the hospital for the purpose of biting the volunteers. The mosquitoes were noted as missing at the time, and the onset of the disease coincided almost exactly in the three cases. It is probable therefore that all the mosquito-transmission experiments really represent the same strain of virus, and in the analysis of cases they have been so treated. As the six cases not actually known to be due to the Collins strain of virus differed in no way from those of the larger group, no significant error can have been introduced by their inclusion.

The presentation of the clinical analysis of our experimental cases will be preceded by a discussion of the symptomatology of dengue as deduced from the literature and from personal observation by Maj. Philip C. Riley, who is particularly qualified for this task by a wide experience with dengue, both in the Philippines and in Mexico. Major Riley has also contributed some observations with regard to the treatment of the disease. The analysis of the symptomatology of the experimental cases and the deductions drawn therefrom are the work of the members of the research board.

SYMPTOMATOLOGY OF DENGUE[12]

Dengue is an acute, febrile, mosquito-borne disease of unknown specific etiology, endemic and epidemic in the Tropics, and at times epidemic in temperate regions during the summer and early autumn. Typical cases are characterized by sudden onset with physical weakness, headache, postorbital pain and soreness, flushing of the face, suffusion of the eyes, anorexia with loss of the sense of taste, backache, pain in the bones and joints, marked prostration, mental depression, and a general feeling of wretchedness. Temperature elevation is coincident with the onset, subsiding on the third or fourth day with amelioration of all symptoms, followed typically by a secondary rise after an interval of twenty-four to seventy-two hours, and accompanied by a recrudescence of pain and distress. This secondary rise of temperature lasts twenty-four to forty-eight hours and usually terminates abruptly. With or slightly before the initial rise of temperature, there is usually a primary erythematous skin eruption best seen over chest, back, sides of trunk, and the flexures of the arms and legs, with a marked tendency to dermographia. There is leucopenia with relative reduction of the polynuclear cells and increase of the lymphocytes. Coincident with the subsidence of the temperature there is a secondary or terminal eruption of a polymorphous character.

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[12] The section on symptomatology, pages 171 to 179 is contributed by Philip C. Riley, major, Medical Corps, United States Army.

In endemic centers the disease is constantly present, perpetuating itself by attacking permanent residents recurrently, often in very mild form. It shows a seasonal variation in prevalence which appears to parallel the variations in numbers of the mosquito vectors. Even in endemic areas, unusual numbers of cases occur from time to time, even amounting to epidemics. In temperate regions in which the winter conditions are such as to preclude the possibility of *Aëdes* living from one season to the next, the disease is characteristically epidemic in type, and attacks a large fraction of the population with an explosive violence surpassed only by that of influenza.

Prodromal symptoms are usually absent, the onset being dramatic in its suddenness. In young children the onset is often accompanied by convulsions. When prodromes appear they are mild in character, consisting of chilly sensations, slight headache, pain in the lumbar region, lack of appetite, and recurring feelings of weakness or weariness. With the actual onset the skin of the face, head, and neck is deeply congested, frequently of a livid color. The face appears swollen. The eyes are suffused and injected. The resulting expression of the face is characteristic, the patient appearing dull and stupid, with obliteration of the natural lines of expression, thus simulating the appearance of an individual recovering from an acute alcoholic debauch. Headache, lumbar pain, and postorbital pain and tenderness on movement of the eyes are an ever-present triad. The headache is frequently violent, it may be generalized, but is usually located in the frontal region or deep behind the eyes. Lumbar pain, with pain in the muscles, bones, and joints is frequently of such severity as to prevent all movement. Bone and joint pains are often described as burning or boring, and are worse at night. Mental depression is frequent and severe, the patient losing all interest in his surroundings and lapsing into a gloomy stupor. Vertigo and nausea are common and are increased by movement. Pain in the testicles and groins has been noted. Abdominal pain and tenderness are common.

The initial skin symptoms are constantly manifested but, as they are evanescent, they must be carefully sought for. These consist typically of an erythematous mottling of the chest, back, inside of the arms and thighs, the plantar and palmar surfaces, and the back of the neck to the hair line. With this mottling, there is present a marked tendency to dermographia. These appearances may occur as early as twenty-four hours before the onset of fever and are considered as pathognomonic when observed. A slight elevation of temperature may exist some hours before the onset of subjective symptoms. The temperature rises rapidly on the appearance of headache, backache, and postorbital pain, reaching its height, 102 to 105° F. or more, within twenty-four hours. It remains high for twenty-four to forty-eight hours, and then in many cases falls rapidly to normal at the end of the third or fourth day, with disappearance of symptoms and without subsequent rise. More characteristic, however, is the so-called "saddle-back" temperature curve, which may be predicted on the failure of the temperature to reach normal after the preliminary rise. In these cases a remission only occurs, followed after one or two days by a recrudescence of the fever and return of all symptoms. Such a secondary rise may be delayed until the seventh or eighth day of the disease. After a day or two more the temperature and symptoms again subside, frequently very rapidly. Free sweating occurs as the temperature descends and is most prominent in those cases in which a crisis is simulated. It appears probable that cases may occur without demonstrable rise of temperature, manifested only by headache, backache, postorbital pain and tenderness, and prostration. Such cases are, of course, not common and are recognizable only during a period when dengue is prevalent.

The secondary skin rash usually makes its appearance with the subsidence of the temperature and is of a macular, erythematous, morbilliform, scarlatiniform, or petechial character. It has even been described as purpuric. The outbreak of the rash is frequently preceded by the most intense itching and burning in the hands and feet, with a generalized sensation of formication. The rash is best seen on the chest, back, back of neck to the hair line, the knees, the dorsum of the foot, and the base of the thumb. In many cases there is seen a characteristic reticulated or lacelike pattern, an intense pink staining of an erythematous nature with "white islands" of sound skin standing out in sharp contrast and conveying the impression that the pink areas are the normal and the white the abnormal. Desquamation occurs more frequently than published descriptions would appear to indicate. It is seen most commonly on the hands and feet, but in some cases is generalized. It begins on the fifth to the seventh day and lasts from five to seven days. The duration of the eruption is extremely variable. In some cases it is so evanescent as to escape notice entirely except on close observation. In one recent case a well-marked maculopapular eruption of considerable extent made its appearance and disappeared completely in the course of thirty minutes. On the other hand, the eruption may persist three or four days, fading gradually and leaving a brownish pigmentation. Some writers have described a purpuric modification of the terminal rash. The nearest approach to this seen by me was an occasional petechial rash which failed to disappear upon pressure.

The tongue is coated centrally, with clear reddened edges and tip. Cyanosis of the face is at times rather intense. Appetite is variable, being generally lost, and there may be an actual loathing of the idea of food. Nausea and vomiting are frequent early symptoms and are aggravated by movement or on the assumption of the erect posture. A rather characteristic early symptom, sometimes persisting for several days, is the loss or perversion of the sense of taste. The patient may describe the condition as a total loss of taste sensation or may say that everything tastes the same. Jaundice may occur but is rare and of a transient nature only. Mild constipation is frequent. An initial or terminal diarrhœa may occur, but is not characteristic. Loss of weight usually results from the attack.

The pulse varies but little with the rise of temperature, though it may be rapid with onset. More commonly it is slow in proportion to the degree of fever. This bradycardia tends to increase with the duration of the disease and is pronounced in convalescence, pulse rates as low as 38 having been noted. The urine is concentrated and reduced in amount. Albuminuria has been noted only rarely by me but has been described as occurring frequently on the second day. When present it is of mild degree and of short duration.

Epistaxis is said to be frequent and, when it occurs, to bring great relief from headache. This symptom has not occurred in the Manila cases. Menstrual disturbances are frequent, free bleeding being common, even in patients well past the menopause.

Glandular involvement varies greatly in frequency according to published reports, but in my opinion is a most constant symptom. The post-cervical, epitrochlear, and inguinal glands are found constantly enlarged, frequently tender, and in some of the cases, with a prolonged subfebrile temperature, the glands have been noted to continue palpable. Tenderness of the inguinal glands has been noted as a prodrome. Enlargement of the spleen and liver has been reported as frequent, but has been noted in but two cases coming under my observation.

Swelling of the joints or joint involvement, manifested by redness or swelling, has not been noted, but is reported by others as being frequent. Joint pains and neuralgic pains in the legs are common and may be prolonged some weeks into convalescence.

Photophobia is common and may precede all other symptoms. Itching and paræsthesia are of frequent occurrence. All types of mild mental confusion may exist during the period of elevation of temperature, cases being noted as drowsy, indifferent with tendency to stupor, or responding with difficulty to questions. Vertigo is a common early symptom, frequently associated with nausea. Both are aggravated by movement or change of posture to the upright position. Absence of abdominal reflexes with exaggeration of the knee jerk has been reported. The characteristics of the white-cell count have been mentioned. Eosinophilia has also been reported, but is probably due to complicating infestation by animal parasites.

Complications are unusual. Pyogenic infections of the skin, pericarditis, orchitis, and inflammation of Cowper's glands have been described. Nonspecific reduction of resistance to infection perhaps plays a part here in allowing the activation of latent infection. Eye complications are relatively frequent and may appear so late as to be considered rather as sequelæ. Catarrhal ophthalmia, keratitis, paralysis of accommodation, and acute glaucoma have been described. Patients frequently complain of blurring of vision and pain when attempting to use the eyes, without adequate discoverable cause; this condition may persist some weeks into convalescence. A peculiar condition reported is that all objects seem to have a yellow flamelike color. The general hæmorrhagic tendency, evidenced by bleeding from mucous membranes, hæmatemesis, purpura, etc., emphasized by some authors, has not been noted by me.

Some degree of involvement of the myocardium is common and should be watched for. Endocarditis has been reported.

Of sequelæ, the eye condition already noted; continued muscular pain and weakness, especially in the legs; mental depression, sometimes marked; and itching of the hands and feet, especially at night, are seen in a considerable proportion of cases. There is also recognized a tendency to dental infections, such as exacerbations of pyorrhœa or root inflammations in the weeks immediately following an attack of dengue. These sequelæ, though not of a very serious nature, do suffice to reduce to a marked degree the efficiency of those affected, and should be considered in any attempt to estimate the amount of disability caused by the disease.

Relapses and recurrences are not uncommon. The nature and degree of the immunity conferred by an attack of the disease are considered at length elsewhere in this report.

Differential diagnosis of dengue is usually easy. However, in the presence of an epidemic, or in localities where the disease is endemic, other acute infections, especially in their early stages, are frequently miscalled dengue, and the possibility of confusion should be borne in mind. Acute infections of the upper air passages, and especially influenza, are perhaps more frequently thus considered dengue in districts where the latter prevails than is the case with any other class of diseases. However, I recall two cases which I, acting as receiving officer of a hospital, admitted as dengue, which subsequently proved to be in the initial stages of acute gonorrhœa. The list of diseases that may be thus confused with dengue is a long one and, though in most cases the confusion is soon brought to an end by the natural evolution of the disease, it is perhaps worth while to mention points of value in the differentiation of early cases. The list includes typhoid fever, yellow fever, trench fever, pappataci fever, typhus, measles, scarlatina, roetheln, cerebrospinal meningitis, plague, influenza, malaria, syphilis, acrodynia, endocarditis lenta, rheumatic fever, the initial fever of smallpox, and acute infections of the upper air passages.

The mortality associated with yellow fever and the fact that it is transmitted by the same mosquito as is dengue, and therefore may spread in any locality where dengue is prevalent, renders the early differentiation of the two conditions particularly important; in early mild cases of yellow fever differentiation may prove for the time impossible. Main dependence in such cases must be placed upon the eye and skin symptoms so characteristic of early dengue. The variability of the leucocyte count in yellow fever, which tends to show a leucopenia in mild cases, renders this method of differentiation of little value. In more-advanced cases of yellow fever the jaundice, albuminuria, and hæmorrhages should prevent confusion.

In the Tropics typhoid fever is often encountered in atypical form. In this condition, too, the blood count is of little differential value. The absence of the distinctive eye and skin symptoms of dengue, together with the results of bacteriologic examination, will soon render the diagnosis evident.

Trench fever presents many points of similarity to dengue, but is distinguished by the tenderness and enlargement of the spleen, leucocytosis, hyperalgesia of the shins, and absence of the skin symptoms of dengue.

Pappataci fever may be impossible of differentiation from dengue in sporadic cases. Careful consideration of the history, search for the punctate red lesion of the sand-fly bite, and absence of the secondary temperature rise and of the rash will assist in the solution of the problem. Experience combined with careful observation will, however, prove to be the best guide.

In typhus the greater prostration, delirium, leucocytosis, and character of the skin manifestations should serve to make the diagnosis clear. However, in the mild cases encountered in Mexico, and there known as "tabardillo," confusion is easy in the absence of laboratory facilities.

The possibility of confusing the initial stages of smallpox, with its high fever, general pains, preliminary eruption, and leucopenia, with dengue should always be borne in mind, especially when dealing with an unvaccinated population. Secondary syphilis, too, when presenting some degree of fever may prove temporarily confusing. Mumps should ordinarily cause no difficulty, but two instances are recalled in which dengue patients with marked glandular enlargement were sent into isolation as suffering from mumps. Of the other diseases mentioned as being more or less difficult to differentiate from dengue, suffice it to say that, if the possibility of confusion be borne in mind, no difficulty should be experienced in their separation, either through characteristic early signs or symptoms or through their nature becoming evident in the natural evolution of the condition.

Of differentiation among the group of so-called "denguelike diseases," including Van der Scheer's fever, six-day fever of Panama, red fever of Caracas, Rogers's seven-day fever, and others, little can be said. It would appear that among themselves they differ little if any more than do different outbreaks of dengue and, until specific etiologic evidence is available, the possibility of their essential identity must be borne in mind.

The prognosis of uncomplicated dengue is good and the mortality negligible, though in widespread epidemics it may not be nil. Deaths have been reported at the extremes of life. In the aged, rapid hyperpyrexia, œdema of the lungs, failure of the right heart, hæmatemesis, purpura, persistent vomiting, nephritis with suppression of urine, and myocarditis have been recorded as causing fatal termination. In children a bad prognosis accompanies the development, at about the fifth day, of rapid cardiac decompensation with collapse, vomiting, and hyperpyrexia. This is also true of hæmatemesis and convulsions. The aged, and sufferers from tuberculosis, diabetes, and chronic bronchitis, chronic alcoholics, and opium smokers are said to be especially liable to fatal termination if attacked by dengue.

The morbid anatomy of dengue is little known, owing to the infrequency of opportunity for investigation. Scheube (1910) states that the following have been observed post mortem: Hyperæmia of the lungs and cerebral meninges with serous exudation into the pia mater; serous exudation about isolated joints; effusion of serous fluid into the pericardium; and softening of the myocardium. I once had the opportunity of performing an autopsy upon the body of a man who, while suffering from a dengue attack, had been killed by his wife. The examination was made in Mexico under unfavorable conditions and for medico-legal purposes. The only abnormality noted in this case was a general marked enlargement of the internal lymph nodes. There was no enlargement of the liver nor of the spleen.

In the treatment of dengue two desiderata are to be borne in mind; namely, the promotion of the comfort of the patient during the attack and the reduction of the proportion of cases showing partially disabling sequelæ. Both these objects are best attained by insistence on rest in bed during the whole course of the disease, including the period of remission. The diet during the first twenty-four to forty-eight hours, or if vomiting be present, should be liquid, but with subsidence of the temperature soft or even full diet may be allowed. Alcoholic drinks should be withheld. Active purgation has proved to be a mistake, though the exhibition of mild aperients may be indicated. Sponging and cold applications are valuable in hyperpyrexia. Aspirin and other salicylates have not been found of use by me, though acetphenetidin has proved very satisfactory. At times hypodermic injection of morphine or codein may be required for the relief of intense pain. Lumbar puncture has been found to relieve intense headache and restlessness, and it may be noted that in a recent series of six cases the fluid withdrawn, while crystal clear, showed evidence of being under increased pressure. Vomiting is best controlled by confining the patient to a recumbent position at all times. It is possible to feed with perfect comfort in this position patients who reject all food as soon as swallowed when in the sitting or semireclining position. Chloral, bromides, and sulphonal have been found useful for insomnia and restlessness. Calcium chloride has been recommended for hæmorrhagic conditions. Collapse in children should be treated by hypodermics of morphine sulphate with measures to combat shock. Attempts to reduce hyperpyrexia usually fail. Skin irritation during or following the rash is best treated by alkaline baths or by sponging. Adrenalin and tincture of belladonna by mouth are said to hasten convalescence.

ANALYSIS OF EXPERIMENTAL CASES

For the clinical analysis, only those of our experimental cases that followed the bites of infected mosquitoes were utilized. The inclusion of cases resulting from successful blood-transmission experiments would have increased the number studied to a considerable degree, but their inclusion does not seem justified in a clinical study, as in them the disease was not acquired in a natural manner. On the other hand, the fact that the mosquitoes used were infected purposely rather than accidentally can have no relation to the symptomatology of the cases resulting from their bites, and we feel that the study of these cases will serve not only to prove the point that the disease with which we were dealing was in fact dengue, but also that certain points in the clinical history of the disease may be brought out very clearly by the study of a fair-sized group of cases, which resulted from the same strain of virus but, otherwise, were entirely unselected. Textbook descriptions of this disease, like those of most others, incline to emphasize typical conditions and seldom give any exact idea as to the frequency with which any symptom is likely to be observed in a given series. Our group of forty-eight cases is large enough to render averages of some significance and to permit of mathematical treatment of the figures along accepted statistical lines. We recognize the fact that dengue, like most infections, varies from time to time in virulence. It seems entirely probable that the general run of cases as seen in an endemic center such as Manila is less severe than it is during an epidemic invading a relatively new area. Accounts of outbreaks of the disease among our soldiers in the early days of American occupation seem to indicate that the average case at that time was more severe than it is at present. Whether this is indeed the fact, or whether the impression is due to the unconscious selection of the severer cases for remembrance on the part of the older officers, cannot be determined at this time. Our series contained a few severe cases, in which the pain and discomfort were sufficient to fit the description of the classical case. Many were of moderate severity and a few presented objective and subjective symptoms so slight as to raise a possible question as to whether or not they should be considered as positive results. Fortunately, in all cases where this question arose it proved possible to answer it definitely by means of further transmission experiments. If a doubtful case is bitten by *Aëdes* mosquitoes shortly after onset and another case arises as the result of the bites after the appropriate interval of "maturation" of the virus, and, if the second case presents unmistakable evidences of dengue, the nature of the original case is unassailably established, provided that the experiment was undertaken under the controlled conditions obtaining in our experimental ward. This method of confirmation was not intentionally used in any case but resulted incidentally to the routine transmission work. All cases considered positive were so declared after consultation with Major Riley, and on clinical grounds alone. The subsequent study of the cases, however, showed that some of them were so mild or varied in some particular from the typical to such a degree as to render a written description of the case unconvincing to one inclined to be skeptical of results. As already stated, all such cases were definitely confirmed by further transmission experiments. Therefore, it may be regarded as established that all of the forty-eight cases here studied were in fact examples of the same disease, and that such variations in character and degree as are shown in their symptomatology reflect the variations that actually occur in naturally acquired cases. The occurrence among them of an occasional case so mild as hardly to attract the attention of the patient himself is highly important epidemiologically, as it constitutes for dengue a confirmation of the assumption often made for other diseases; namely, that the disease may be spread and the virus carried over periods of apparent absence by means of missed cases of extreme mildness. To carry this a step farther, it appears possible that cases may occur of such mildness as to render their recognition on clinical grounds alone impossible. The study of individual symptoms and their variations suggests this possibility, and the point will be brought out later in the discussion.

The first step in our analysis of these cases consisted in the preparation of complete abstracts of the clinical histories of the cases, covering the period of isolation or observation as well as that of illness. All volunteers were under close observation during their stay in the experimental ward, and regular temperature records were kept. On the development of clinical dengue, the men were transferred to the regular hospital wards and the required official records made by the ward surgeons. Major Riley was responsible for the completeness and accuracy of these records, and to his interest and industry we are indebted for much of the value of the results. With the abstract from the clinical history of each case a plot of the temperature record from the time of admission to the experimental ward until final discharge from the hospital was also prepared. With these as readily accessible sources of information, the symptomatology of the series was then tabulated by cases, each symptom being recorded as present or absent, and information briefly entered as to character and time of appearance of rash, degree and duration of temperature, character of leucocyte counts, etc. The basic table thus obtained contained the essential clinical history of each individual case and proved to be so large and of so unwieldy a nature as to render its reproduction impracticable. A consolidated tabulation of the symptoms shown by the group as a whole is accordingly here presented to show in general the results of the clinical study. Certain special features will be developed more fully later. For comparison and control a similar tabulation of the symptomatology shown by a series of twenty-four naturally acquired cases, occurring consecutively during the period covered by our work, is also shown. (See Tables 21 and 22.)

TABLE 21.—*Symptomatology shown by experimentally induced dengue cases.*

Symptom.	Noted as present.		Noted as absent.		Not noted.	
	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Prodromal symptoms	31	65			17	35
Chilliness at onset	15	31			33	69
Sudden onset	14	29	2	4	32	67
Headache	44	92	1	2	3	6
Postorbital pain and soreness	33	69	3	6	12	25
Flushing of face	38	79	3	6	7	15
Injection and suffusion of eyes	37	77	1	2	10	21
Characteristic facial expression	15	31			33	69
Backache	16	33			32	67
Pains in limbs	12	25			36	75
Generalized pain	15	31			33	69
Anorexia	20	42	1	2	27	56
Loss or perversion of sense of taste	18	38	5	10	25	52
Nausea	17	35	8	17	23	48
Vomiting	4	8	9	19	35	73
Vertigo	24	50	4	8	20	42
Nose bleed			9	19	39	81
Other hæmorrhages					48	100
Adenopathy	37	77	6	12	5	11
Congestion of throat	9	19			39	81
General malaise	23	48	25	52		
Diarrhœa	1	2	47	98		
Peak temperature reached within 24 hours	25	52				
Peak temperature reached later	23	48				
Peak temperature in second wave	5	11				
Saddle-back temperature curve	19	40				
Near saddle-back temperature curve	14	29				
Single-wave temperature curve	15	31				
Rash with onset	37	77	6	12	5	11
Terminal rash	40	83	8	17		
Leucopenia						

TABLE 22.—*Symptomatology shown by naturally occurring dengue cases.*

Symptom.	Noted as present.		Noted as absent.		Not noted.	
	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Onset sudden	18	75	1	4	5	21
Chill with onset	10	42	4	16	10	42
Headache	24	100	------	------	------	------
Postorbital pain and soreness	22	92	------	------	2	8
Flushing of face	16	67	3	12	5	21
Injection and suffusion of eyes	10	42	------	------	14	58
Characteristic facial expression	6	25	1	4	17	71
Backache	14	58	1	4	9	38
Pains in limbs	11	46	------	------	13	54
Generalized pains	6	25	------	------	18	75
Anorexia	14	58	------	------	10	42
Loss or perversion of sense of taste	13	54	5	21	6	25
Nausea	8	33	12	50	4	17
Vomiting	2	8	16	67	6	25
Vertigo	21	88	1	4	2	8
Nose bleed	------	------	16	67	8	33
Other hæmorrhages	------	------	------	------	------	------
Adenopathy	20	83	------	------	4	17
Congestion of throat	7	29	1	4	16	67
General malaise	22	92	------	------	2	8
Diarrhœa	2	8	13	54	9	38
Rash with onset	14	58	5	21	5	21
Terminal rash	15	62	------	------	9	38
Leucopenia	21	88	3	12	------	------

A comparison of the figures shown in the two tables reveals the essential similarity of the symptomatology shown by the two groups of cases. Differences in the proportion of cases in the two groups manifesting certain symptoms are of interest and significance. The group of experimentally induced cases was under entirely different environment from that of the group of naturally acquired ones up to the time of admission to the hospital. The experimental cases were confined to the ward and consequently at rest. The cases from outside came from duty and in most cases were actively at work up to the time of admission. As it will be shown later that this group as a whole had evidently been suffering from the disease for more than a day before reporting at the hospital, it is natural to attribute the greater prominence of some symptoms, such as vertigo and malaise, to the fact that the men continued at work after the disease had started. Another result of the presence of the experimental cases in the observation ward was the possibility of watching them carefully for early or prodromal symptoms.

The onset in the experimental cases is stated to have been sudden in fourteen of the forty-eight cases, or 29 per cent; it was gradual in 4 per cent, and in the balance this point was not noted. We find, however, that in thirty-one, or 65 per cent, of the cases, prodromal symptoms were recorded by the nurse in the observation ward. This high proportion runs counter to the usual description of the disease and is explained by the unusual opportunity of watching the cases develop. Such prodromal symptoms usually preceded the rise of temperature by from six to twelve hours, but in one case they were evident for two days before the advent of fever. Headache, weakness and malaise, sleepiness, backache, sore eyes, and loss of appetite were the usual complaints.

With the rise of temperature all the symptoms were aggravated. The headache, postorbital pain and soreness, flushing of the face, and injection or suffusion of the eyes were very constantly present and, together, led to the characteristic facial expression described by Major Riley. Backache, pains in the limbs, and generalized pain, sufficiently intense to be complained of by the patient, were somewhat less common in the experimental cases than in the symptom group first mentioned. In the naturally acquired cases they were rather more prominent. Loss of appetite was noted in about half of the cases and not particularly noted in most of the rest. In one case it was stated that appetite was not lost. The interesting nervous disturbance evidenced by loss or perversion of the sense of taste is prominent in both groups of cases. The men complained that food had no taste or that all articles of food tasted the same. In some cases an unpleasant taste persisted in the mouth independently of food. Nausea was a complaint in about a third of the cases, leading to vomiting, however, in only about a quarter of the cases so complaining. Vertigo was still commoner than nausea; it occurred in half of the experimental cases and in nearly 90 per cent of the cases from outside. General malaise of such degree as to require special notice occurred in nearly all of the natural cases but in less than half of the experimental group.

Hæmorrhage from the nose or elsewhere was not noted in either series. Adenopathy was noted in about 80 per cent of each group. This interesting condition would appear to be among the most characteristic and constant evidences of infection with the virus of dengue. In one case personally observed enlargement and distinct tenderness of the right epitrochlears preceded the onset by two days. Major Riley mentions the occurrence of tender inguinal glands before onset. Taken in connection with the biting habits of *Aëdes* mosquitoes, which seem to prefer the ankles and elbows as points of attack, these observations may indicate the occurrence of a primary adenopathy in the lymph nodes that drain the area of the infecting bite. Observation of our experimental cases for this point was entirely negative. Diarrhœa and cough, which have been described as sometimes present in dengue, did not occur in our cases more frequently than might be expected in any similar-sized group. Congestion or injection of the mucous membrane of the throat occurred in 19 per cent of the experimental cases and in 29 per cent of those that occurred spontaneously. In the remaining cases of each group this point was not specially mentioned in the records. As this symptom appears rather a localization of the primary eruption than a manifestation of a local inflammatory process, and as it is seldom accompanied by discomfort in the throat, its occurrence is probably overlooked in many instances.

Comparison of the symptomatology shown by the experimental cases of our series with the classical description given by Major Riley serves to convince one that the disease with which we were dealing was in fact dengue. Certain further evidence is available, however, in the character and duration of the temperature curve and in a study of the white blood counts and of the eruptions. These features of the symptomatology, together with the duration of the period of incubation, in as much as they are expressed quantitatively, may be studied by statistical methods and certain conclusions drawn therefrom that appear of more than passing interest.

The period of incubation.—The incubation period of dengue has been variously given. The clinical observations bearing on this point are collected in the section of this report dealing with the history of the disease. Cleland, writing in Byam and Archibald's Practice of Medicine in the Tropics (1923), concludes that the evidence on hand indicates that the incubation period is usually from three to eight days, with extremes of two and a half and fifteen. The shorter periods referred to appear to have been based on cases induced by the intravenous inoculation of the virus. The extreme period of fifteen days is based on the observation of a case of blood transmission reported by Cleland and Bradley (1919). The protocol of that case shows nothing to criticize and the observation must be accepted, at least for cases induced by the injection of infected blood;

the blood used for the case was drawn from the infected donor late on the fourth day of the disease (ninety hours). Our experimental series of cases induced by the bites of infected mosquitoes provided an unselected group of cases, infected by the natural route, in which the lapse of time between the infective biting and the onset of fever (which we have regarded as the definite beginning of the disease) is definitely known. In the protocols of these cases the incubation period has been recorded to the nearest quarter day. If these observations be grouped the distribution shown in Table 23 and plotted in fig. 15 is obtained. The figures are combined in groups of whole days to avoid the inequalities due to the small number of observations and to obtain a fairly smooth curve.

TABLE 23.—*Showing the frequency of incubation periods in experimental dengue.*

Days.[a]	Cases.	Per cent.
4 to 4.99	12	25
5 to 5.99	17	35
6 to 6.99	7	15
7 to 7.99	7	15
8 to 8.99	3	6
9 to 9.99	1	2
10 to 10.99	1	2
Total	48	100

[a] Mean, 6.05 days.

The greatest number of cases (seventeen, or 35 per cent of the group) fell on the fifth day after biting. Twenty-five per cent had an incubation period of over four and less than five days, and the sixth and seventh days showed 15 per cent each. The shortest incubation period observed as the result of mosquito-borne infection was four and a quarter days, while the longest was ten days. The curve obtained by plotting the data (fig. 15) is, of course, not symmetrical, the variations being much wider on the side of the longer periods than on that of the shorter ones. However, for the purpose of estimating the probability of variations that tend to be extreme, such as the fifteen-day incubation period recorded by Cleland and Bradley (1919), it is permissible to consider only the trend of the curve that records the frequency of periods above the mean. If this be done, the probable error of a symmetrical distribution with a curve of the same shape will be found to be one and three-tenths days.

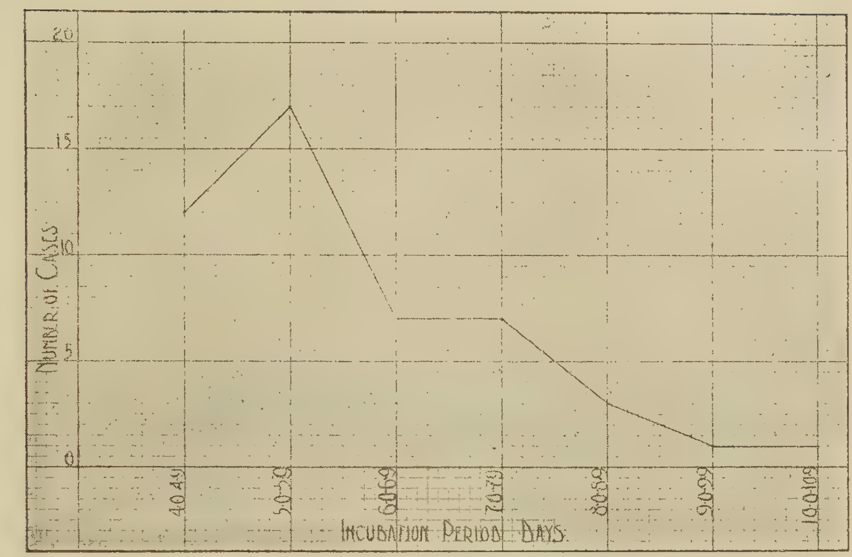

FIG. 15. Chart plotted from the data in Table 23, showing frequency of incubation periods by days in experimental series of forty-eight cases of dengue.

Our extreme observation, ten days, is thus seen to differ slightly over three times the probable error from the mean (6.05 days) so that according to chance it should occur once in about twenty-five times. That one such case should have occurred in forty-eight observations is then exactly what should be expected. An incubation period of fifteen days, however, is a deviation over seven times the probable error, and the odds against such an occurrence in a group of cases showing the distribution of incubation periods given here are several hundred thousand to one. It is manifestly impossible, with no basis other than this analysis, to deny the possibility that such a case actually may have occurred; but, obviously, another case showing so long an incubation period is not to be expected.

The average incubation period in our series was six and one-twentieth days; in 25 per cent it was less than five days; in 60 per cent, less than six days; in 75 per cent, less than seven days; and in 90 per cent, less than eight days. Incubation periods of more than ten days must be very rare in naturally acquired cases.

The fever.—Some interesting generalizations with regard to the character and duration of the fever are possible from the figures provided by the experimental series. The highest point reached by the temperature during the course of the disease is shown in Table 24 and plotted in fig. 16.

Table 24.—*Highest temperature reached during course of attack of experimental dengue.*

Temperature.	Cases.	Per cent.
°F.		
99.5 to 100.4	5	10.5
100.5 to 101.4	17	35.4
101.5 to 102.4	13	27.1
102.5 to 103.4	9	18.7
103.5 to 104.4	4	8.3
Total	48	100.0

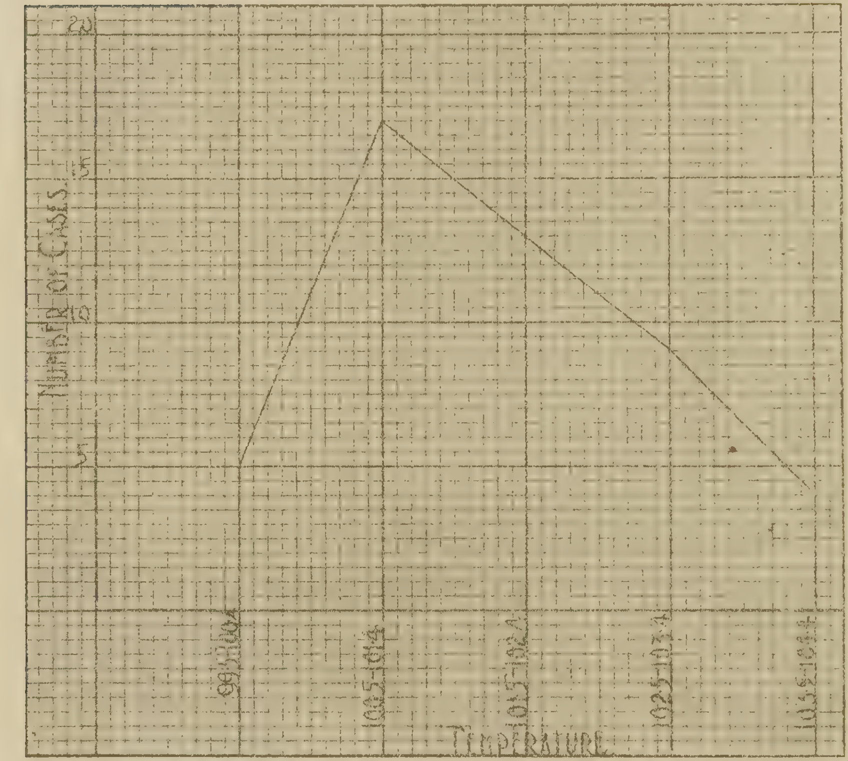

Fig. 16. Chart plotted from the data in Table 24, showing the highest temperature reached in the forty-eight experimental dengue cases.

The curve in fig. 16 is only moderately asymmetrical and suggests a fairly even distribution about its mean of 101.9. The highest point reached in the case with the lowest maximum temperature was 99.6°. The highest temperature observed in the group was 104°. Inspection of the temperature curves of the cases previous to the development of dengue shows that temperatures of over 99° and occasionally over 100° are common in health, under the conditions of the experiment. Balfour, in Byam and Archibald's Practice (1921), calls attention to the more or less regular occurrence of slight temperature elevations in unacclimatized whites in tropical regions. Thus, to base a diagnosis of dengue infection on a temperature of 99.6° would appear to be unjustified. The case in question (Working A–57, of the experimental series) was in all other respects a typical case of dengue. His subjective symptoms were definite, he showed a characteristic macular rash on the third day, and he had a moderate degree of leucopenia. More convincing still, mosquitoes allowed to bite him on the first day of his illness were shown to be capable of transmitting the disease to others. The degree of fever attained by a given case of dengue is thus seen to be very variable, and in at least one proven case is shown to be hardly above normal. This suggests the possibility of cases occurring in which fever may be altogether absent. If such do occur it is important from the standpoint of the epidemiology and prevention of the disease. The study of the duration of fever in our series furnished further suggestive evidence in this direction.

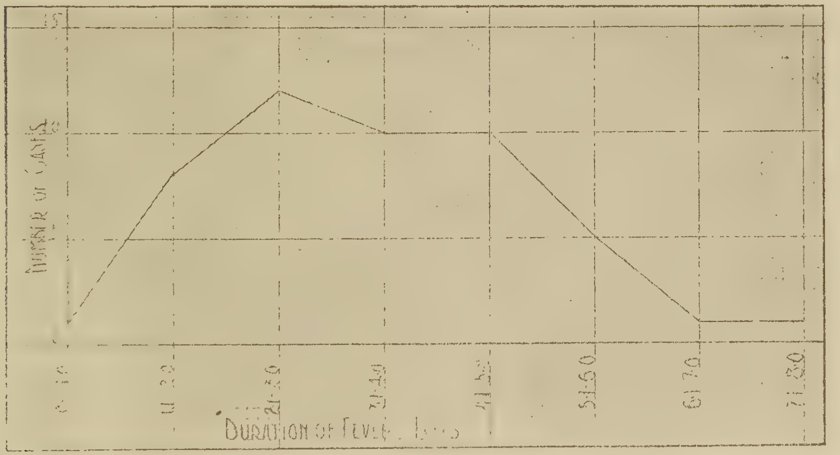

FIG. 17. Chart plotted from the data in Table 25, showing duration of temperature in the forty-eight experimental dengue cases.

The data shown by the study of the duration of temperature in our cases are grouped in Table 25 and shown graphically in fig. 17. The curve is more symmetrical than that in fig. 16 and shows a fairly even distribution about the median of 3.3 days. The mean, affected more by the cases of long duration, is 3.92. In Table 24, which records the highest temperature reached, we

TABLE 25.—*Duration of fever, onset to final defervescence, in experimental dengue.*

Duration.	Cases.	Per cent.
Days.		
0.1 to 1.0	1	2.1
1.1 to 2.0	8	16.6
2.1 to 3.0	12	25.0
3.1 to 4.0	10	21.0
4.1 to 5.0	10	21.0
5.1 to 6.0	5	10.5
6.1 to 7.0	1	2.1
7.1 to 8.0	1	2.1
Total	48	100.4

found a small number of cases with practically no fever. In the present tabulation it is seen that one case had only one day's duration of fever; however, the two observations do not represent the same case. The two curves agree in that both suggest that, under the laws of chance, cases probably will occur in which the degree of fever and its duration will be reduced to the vanishing point and afebrile dengue will result. Had such a case occurred in our experiments it would have been regarded as a negative attempt at transmission and would not have been included among the forty-eight successful cases. Therefore, it becomes of interest to investigate the records of the cases regarded as unsuccessful to see if any evidence exists in them that might indicate the possibility of afebrile dengue. Such evidence must be sought among the records of men bitten by mosquitoes known to have been infective, or at least by mosquitoes known to have bitten infected individuals a sufficiently long time previously to have rendered the insects infective. If a man bitten by such mosquitoes should show suspicious symptoms after the usual period of incubation, and on later inoculation with infective blood should prove to be immune, the suspicion of an attack of afebrile dengue would become a probability.

Our records show three cases of this type, and the point at issue is perhaps of sufficient theoretical importance in relation with analogous cases in other infections to warrant their discussion here.

Judy, Maurice (A–49, A–56, B–13).—This man had been in the Islands only one week. After the usual period of observation he was bitten by 21 *Aëdes* infected thirteen days previously, on the first day of dengue. Another lot of mosquitoes infected from the same case (A–40), at the same time, proved capable of transmitting the disease. This biting, however, was ineffective. Ten days later he was bitten by 19 mosquitoes of the same lot. After five days he developed headache, sore eyes and, later, a white-cell count of 4,700, with 58 per cent neutrophiles and 38 per cent lymphocytes. A later attempt to infect him by means of blood was unsuccessful.

Wilson, D. E. (*A-84, A-93, A-99, B-12*).—This man had been bitten by mosquitoes infected on the fourth day of the disease, but with entirely negative results. Ten days later he was bitten by five "second-day mosquitoes" known to be infective (see A-117). After six days he had headache and his eyes appeared red. Temperature reached 99.2°, which is without significance in the Tropics. His eyes remained sensitive for several days, but no other symptoms developed. He then received subcutaneously 0.5 cubic centimeter infected blood, but the disease did not develop.

Carroll, E. H. (*A-95, A-109, B-14*).—After nine days' observation in the experimental ward, this man was bitten by 17 *Aëdes* mosquitoes, infected nineteen days previously from an experimental case one and a half days before the onset of symptoms; the result was negative. Ten days later he was bitten by 16 *Aëdes* mosquitoes, infected twenty-six days previously from the same case, then only a half day before onset. These mosquitoes had been shown by previous successful transmission (see A-96) to be infective. After five days he complained of aching in knees, shoulders, and elbows, but was without fever. On the following day it was noted that his food was without taste. The next day "aches all over, headache, eyes sore when he looks down. Still no fever." The succeeding day he made no complaint, but on the day following it is recorded that he ached all over. Two days later still he was given 0.5 cubic centimeter of citrated blood from A-111, then in the first day of dengue, with entirely negative results.

These observations speak for themselves. There is nothing convincing per se in the observations made on any one of the three cases; but, taken in connection with the known infectivity of the mosquitoes used, with the subsequent demonstration of immunity, and with the probability of the existence of afebrile dengue as suggested by the curves of duration and intensity of fever in the positive cases, these cases may perhaps be admitted as evidence tending to show the probability of dengue without fever. The crucial point in the demonstration, the further transmission of the disease by mosquitoes infected from cases of this character, is lacking, because the cases were regarded as entirely negative at the time and the experiments in progress had no relation to the point under discussion.

Character of the temperature curve.—The typical curve of temperature in dengue has been described by Major Riley. Casual observation of a number of clinical cases in the wards of Sternberg General Hospital had convinced us that the disease as manifested in Manila shows the diphasic temperature of the classical description only rarely. It is of particular interest on that account to note that of our forty-eight cases, nineteen, or

40 per cent, showed characteristic "saddle-back" temperature curves. Fifteen cases, or 31 per cent of the group, had only a single wave of fever. The remaining 29 per cent showed curves of intermediate types which might be described as "near saddle-back" or as suggestive of the diphasic curve. The common occurrence of this type of fever curve, compared to its infrequency in the usual run of dengue as seen in the hospital, calls for comment. Reference to the fever charts of our cases in the Appendix shows that in many cases the primary wave of fever is short-lived, persisting often only one or two days. The mean duration of the fever in the experimental cases has been shown to have been three and nine-tenths days. In the series of twenty-four naturally occurring cases tabulated for comparison, none of which showed a characteristic diphasic temperature curve, the mean duration of fever after admission was only one and nine-tenths days. The difference of two days' duration between the two series, otherwise similar in all respects, would seem to indicate that the average case was not admitted to the hospital until late on the second day of the disease. If this be true it is evident that only rarely is it possible to observe the whole course of the fever in such cases, and the failure to see diphasic temperature curves is explained.

Certain other characteristics of the fever curve may be noted in passing. Twenty-three cases, or 48 per cent of the group, showed a rapid rise of temperature, the peak of the curve having been reached within twenty-four hours of onset. In the remaining 52 per cent the rise was more gradual. In five of the nineteen cases showing a definitely diphasic temperature, the second wave reached a higher point than did the first. The termination of fever was usually by a rather rapid lysis, one and a half days having been required to reach a permanent normal. However, three cases showed a definite crisis, the fever having fallen to normal within a few hours. An interesting point in this connection is that, for several days following defervescence, or as long as the men were under observation, the variations in temperature uniformly observed before the development of dengue were no longer in evidence; the temperature remained almost constant between 98.0° and 98.6°.

The eruptions.—Our experimental cases afforded an unusual opportunity for the study of the eruptions of dengue, especially of the primary or initial rash. It is frequently the case, if indeed it is not the rule, that a patient will postpone reporting to his physician until a time when the evanescent initial rash has disappeared. The evanescent quality of the initial rash has been emphasized frequently by writers on the subject and is well recognized. The fact that our cases were all closely watched for eruption for some time before onset enables us to give a fairly accurate account of the incidence and character of the initial rash. However, the records of only forty-seven cases are available for this purpose, as in one the record of the case after transfer to the regular hospital ward was unfortunately lost. Of the forty-seven cases five, or a little over 10 per cent, showed no discoverable initial rash. It is, of course, possible that still closer observation might have disclosed an eruption in some of these. In three cases the existence of rash was doubtful, an erythematous mottling of slight degree having been present, but it was hardly distinct enough to be convincing. In ten cases the initial rash is recorded as definitely present, but its character is not described. The character of the remaining eruptions observed varied greatly, from one case that showed a definite erythematous flush, to cases that showed intense maculo-papular or measleslike rashes. Seven cases showed a distinct erythematous mottling; eight, a more diffuse erythema. Nine cases showed a rash described as macular and erythematous, and in two the eruption at onset was maculo-papular and in two measleslike. The usual points of election for the location of these rashes have been noted by Major Riley. It may be significant that there is a tendency for the initial and terminal eruptions to occur in general on the same parts of the body. Two of our cases showed a very transient eruption. In nine, on the contrary, there was only one eruption which lasted throughout the course of the disease. These nine included the maculo-papular and measleslike initial rashes, the type of which resembles that of the later eruption. This peculiarity of the eruption bore no relation to the duration of the fever nor to the shape of the fever curve. Four of the cases had short single-wave temperature curves, four belonged to the definitely diphasic fever group, and one was intermediate. The most probable explanation of this condition seems to be that the initial rash persists until the appearance of the terminal, and that the two blend.

If the nine cases described above are to be regarded as examples of initial eruption only, we had seventeen cases in which the terminal eruption failed to appear. In two of these no eruption was observed during the course of the disease; both were confirmed by further transmission of the virus. In six the terminal eruption was not observed, although the records show a short-lived initial eruption; three of these were confirmed by transmission. Three of the nine cases with prolonged initial eruption having the general characters of the terminal rash were also thus confirmed. Thirty cases showed definite terminal rashes. These facts are tabulated in Table 26.

TABLE 26.—*Data with regard to occurrence of rashes in dengue.*

	Cases.	Per cent.
No rash seen at any time	2	4
No discoverable initial rash; terminal observed	7	15
Initial rash prolonged several days and no separate terminal rash	9	19
Transient initial rash with no terminal rash	6	13
Terminal rash seen	30	64
Both rashes distinct and separate	23	49

The day of appearance of the final rash in the thirty cases that showed eruptions which may be definitely classified as terminal is as follows: Second day, 2 cases; third day, 12; fourth day, 7; fifth day, 6; sixth day, 3; and mean, 3.9 days. The mean time of appearance of the terminal rash is seen to coincide with the mean duration of fever in the whole group. Characteristically, then, the appearance of the terminal rash coincides with the final fall of temperature, and a note to that effect was made in a great many of the cases. This rule, however, has its exceptions, and cases were observed in which the terminal rash appeared with the secondary rise of temperature, and others in which the appearance of the eruption was delayed several days into convalescence.

In twenty-seven instances the terminal rash was definitely described. The types of eruption are as follows: Erythematous, 2 cases; macular, 8; maculo-papular, 10; measleslike, 4; scarlatinal, 2; and petechial, 1.

The average duration of the terminal rash was two and seven-tenths days. In the more-marked rashes, disappearance was often followed by a brownish pigmentation which persisted for some time. Owing to prompt discharge of patients and their return to duty on recovery, we have no data with regard to desquamation in our experimental series. The occurrence of itching in connection with the terminal eruption was noted in several cases. This symptom may precede, accompany, or follow the eruption and often persists well into convalescence.

In only two, or 4 per cent, of our cases was there failure to observe an eruption some time during the course of the disease. Six cases showed initial but no terminal rash, and in seven the condition was reversed. Twenty-three showed both initial and terminal rashes in classical manner. In nine the initial rash persisted throughout the disease, and in type resembled the usual terminal eruption. The initial rash usually proved evanescent and was of an erythematous character, while the terminal rashes were of longer duration and frequently showed a papular or, in one case, even a petechial character in addition to the erythematous flush. Evidently the eruption is a very variable symptom, requiring careful and frequent observation if all its details are to be noted; in some cases it may fail altogether to develop.

BLOOD

There is general agreement, in the published studies of the blood findings in dengue, that there are no characteristic changes in the red cells and the hæmoglobin, on ordinary clinical examination. Certain investigators (Graham, 1903; and Eberle, 1904) believed they found cell inclusions in the erythrocytes and they attributed to them the rôle of parasitic cause of the disease. Such findings are more fully discussed in the consideration of the specific etiology of the disease; they have so far lacked confirmation at the hands of others than the original discoverers. It can be safely said that nothing of diagnostic interest is to be found on examination of these elements of the blood by ordinary clinical methods.

There is equally general agreement that there is in dengue a diminution in the number of circulating leucocytes and that the relative proportion of polynuclear neutrophiles is decreased whereas that of the lymphocytes is increased. The blood studies made on the series of experimental cases here under consideration amply confirm the general conclusions reached by previous investigators. However, it has seemed that rarely in the history of the investigation of disease has an opportunity comparable to the present one presented itself for the detailed study of blood changes. We have at hand not only the series of white counts made during the course of the induced disease, but we have a corresponding series of preliminary counts made on the same individuals while under close clinical observation before the disease developed. With these data we are in position to judge whether or not there was a reduction in the count in a given individual (below what is habitual with him), and also to establish a "normal," or control, series with which subsequent series taken during the disease could be compared. These rather unusual conditions have made it seem worth while to subject the figures to a careful analysis in the hope of deducing some general relations, or "laws," which might be of value not only in the diagnosis of dengue but possibly also in the interpretation of blood counts in general.

The whole value of such a study is, of course, very largely dependent on the care with which the individual counts were made. The counts in this series were made by the laboratory force of Sternberg General Hospital, in the course of their regular routine work. For the establishment of the differential count a minimum of 200 cells was counted and the officers in charge of the laboratory, Maj. Cyrus B. Wood and Capt. Milner H. Eskew, both of the Medical Corps, United States Army, state that when the results of the first two consecutive hundreds of cells counted did not substantially agree a greater number was used. Clinical pathologists will probably agree with us that single differential counts are frequently misleading and difficult to interpret. However, when the results of a large number of counts, whether of total cells or of differential relationships, arrange themselves in such manner that they can be plotted out in a characteristic frequency curve, in the same manner as is done in measuring any other biological character, there is strong evidence of the substantial accuracy of the work on which the figures were based. The curves shown in figs. 18, 19, and 20, of the normal and dengue blood counts, as well as of the relationship between the total count and the differential, are grounds for the exhibition of great confidence in the care with which the counts were made.

Normal counts.—At least one preliminary count of leucocytes was made on each case in the experimental series. In most cases more than one count was made, not only for the purpose of furnishing a basis for comparison with later counts during the induced disease, but also as a part of the preliminary clinical examination intended to rule out any men who presented complicating conditions that might interfere in the subsequent interpretation of results. In all, eighty-two such counts were made on the forty-eight subjects of experiment that gave positive results in the mosquito-transmission experiments. The result of these counts is tabulated in Table 27, in the form of a frequency distribution.

TABLE 27.—*Blood counts in experimental dengue cases.*

White blood count.	Cases.
4,000 to 4,999	3
5,000 to 5,999	3
6,000 to 6,999	15
7,000 to 7,999	24
8,000 to 8,999	23
9,000 to 9,999	10
10,000 to 10,999	1
11,000 to 11,999	2
12,000 to 12,999	1
Total	82

This distribution of counts plots out as a fairly symmetrical frequency curve, and can fairly be treated as such mathematically. The mean value of the white blood count in this series is 7,854; the median value 7,812. The probable error of the distribution is 979; that is to say, half of all the observations fall within 979 on one side or the other of the mean. It can be shown that if any observation in such a series differs from the mean by more than three times the probable error (P.E.) the odds are 21 to 1 against its being the result of chance variation in the count. In other words, the odds are 21 to 1 in favor of the assumption that some factor or factors other than those that influence the main body of the distribution have intervened to cause such a wide deviation from the mean. In the establishment of a normal standard, then, a truer value is to be obtained by excluding from consideration those values which are found to be more than 3P.E. different from the mean of the distribution. Applying this correction to the distribution in question excludes all the counts below 5,000 and those above 10,000, seven in all, which reduces the number of counts that can be regarded as normal to 75. These limits are, of course, those usually regarded by clinical pathologists as those of the normal count. Practically two-thirds of the values fall between 7,000 and 9,000. The counts were made during the forenoon, several hours after the regular hospital breakfast. The correspondence of the values found with those generally accepted, together with their distribution in a fairly symmetrical "frequency curve," is accepted as satisfactory evidence of the substantial accuracy of the counts themselves, and makes for confidence in the results obtained in the subsequent counts made during the course of dengue.

The mean value of the series of normal counts, 7,793, after excluding the seven cases outside the 3P.E. limit is only slightly lower than that of the original series; the median value of 7,812 remains unchanged.

Counts in dengue cases.—In the forty-eight cases of mosquito-induced dengue, one hundred fifty-four leucocyte counts were made during the course of the disease and early convalescence, or an average of over three counts per case. Of these forty-eight cases twelve, or 25 per cent, failed to show any diminution in the number of circulating leucocytes when compared with their own preliminary counts. It appears, then, that leucopenia is by no means a constant or necessary feature of this disease and its absence cannot be regarded as negativing the diagnosis of dengue. In the study of the counts obtained on the successive days of the disease, these cases are included in the averages.

Counts at onset.—In eleven cases white-blood counts were had within a few hours of the first appearance of symptoms, and before the patient had been transferred to the regular dengue ward of the hospital. The average value of those eleven counts was 7,750, not significantly different from the normal mean. The highest count observed at onset was 13,500; this occurred in a patient whose two preliminary counts had been below 10,000. The lowest count at onset was 4,900. Of the eleven, two showed counts definitely higher than those before the disease developed, and two showed counts definitely lower. The number of cases is far too small to provide a basis for a general statement, but the observations suggest that normal or somewhat increased numbers of leucocytes found in counts made within the first few hours of the disease are not incompatible with the existence of dengue.

Subsequent counts.—Excluding the eleven counts that were taken shortly after onset, there remain one hundred forty-three counts made during the course of the fever or in early convalescence. These counts are tabulated in Table 28.

TABLE 28.—*White blood counts in dengue (excluding counts made at onset).*

White blood cells.	Counts.	Per cent.
3,000 to 3,999	3	2
4,000 to 4,999	25	18
5,000 to 5,999	54	37
6,000 to 6,999	36	25
7,000 to 7,999	11	8
8,000 to 8,999	7	5
9,000 to 9,999	7	7
Total	143	100
Median	5,811	

These results are plotted in fig. 18, which also gives for comparison the curve shown by the normal counts after the exclusion of the curves that differ more than 3P.E. from the mean. It is seen from this chart that about one-fourth of the area included below the curve of dengue counts falls also within the area inclosed by the normal curve—a graphic expression of the fact already noted that one-fourth of the dengue cases failed to show leucopenia at any stage of the disease. The median values, however, 5,811 and 7,812, are separated by 2,000 points on the scale. In as much as the P.E. of each of these averages is less than 100 it is seen that a very definite leucopenia characterizes dengue. Only 2 per cent, however, showed values below 4,000. The application to this series of the rule of excluding values more

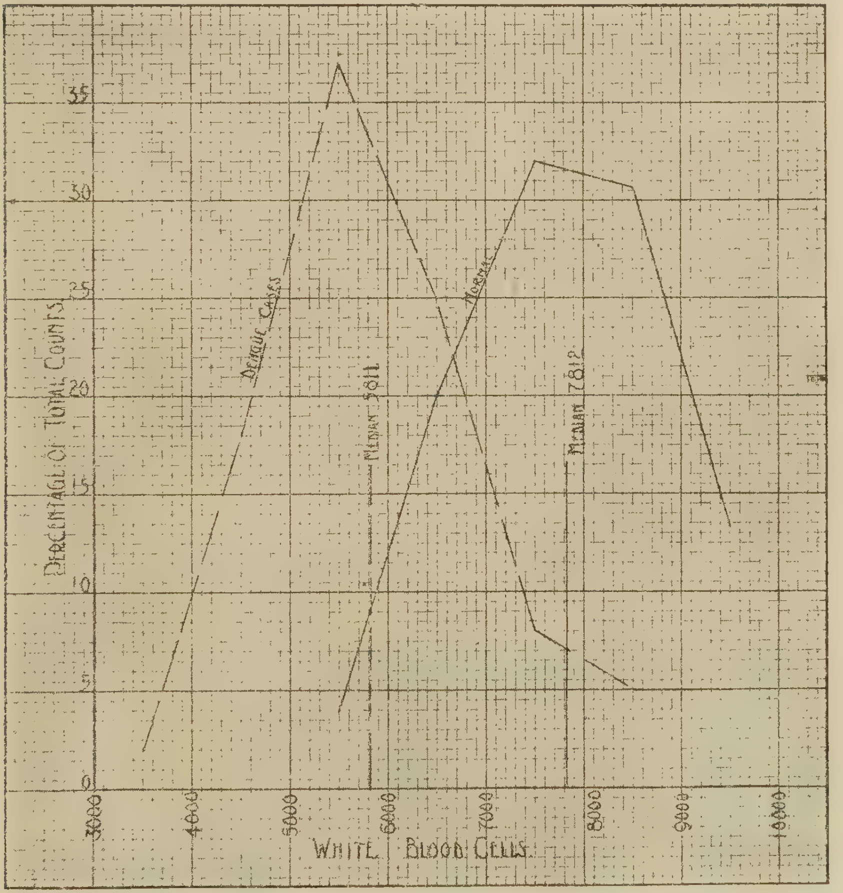

FIG. 18. Chart showing the percentage distribution of total leucocytes in seventy-five normal cases and in one hundred forty-three cases of dengue.

than 3P.E. different from the mean, excludes from consideration counts above 8,650. It can be said, then, that if error in counting and the presence of complications causing leucocytosis can be excluded, the odds are 21 to 1 against the existence of dengue in a case giving a count materially higher than that figure.

In the counts considered separately for each day of the disease the same tendency is shown as for the series taken as a whole. The values found outside the 3P.E. limit are usually high, and in many cases the study of the clinical records yields evidence of septic complications justifying their exclusion from the series. There remain one hundred thirty counts made in dengue cases subsequent to the onset. Of these, twenty-eight were made during the first day of the disease, thirty-six on the second, thirty-one on the third, twenty-one on the fourth, nine on the fifth, and five on the sixth. The small number of counts on the fifth and sixth days, together with the fact that in the majority of such cases convalescence was well established at the time, renders averages on these later days of little value. The results for counts of the first four days are tabulated in Table 29. Each day's counts were first grouped separately and such as fell outside the 3P.E. from the mean for the day were excluded. This resulted in throwing out four counts on the first day, two on the second, and one each on the third and the fourth. This suggests that the results of counts are more apt to show inconclusive results on the earlier days than when made later. Table 29 includes only those remaining after the exclusion of the aberrant counts and therefore better represents the tendency for the day than do the uncorrected figures.

Table 29.—*Dengue counts (excluding those at onset, and those deviating more than 3P.E. from the mean of the day).*

White blood cells.	Counts.				
	First day.	Second day.	Third day.	Fourth day.	Total.
3,000 to 3,999	0	1	1	1	3
4,000 to 4,999	2	7	5	7	21
5,000 to 5,999	10	14	12	8	44
6,000 to 6,999	8	7	10	3	28
7,000 to 7,999	4	2	1	2	9
8,000 to 8,999	2	3	1	0	6
9,000 to 9,999	0	0	0	0	0
Total	26	34	30	21	111
Mean	6,269±139	5,823±137	5,767±123	5,404±150	5,833±71
Median	6,125	5,643	5,750	5,386	5,715

Of the nine counts made on the fifth day, four were from patients definitely convalescent at the time the count was made. They varied from 9,200 to 4,700. One with a temperature of 103° was 4,300; the others fell between 5,000 and 7,000. Of the five counts made on the sixth day of the disease, two patients had slight fever at the time of the count and all counts fell between 4,800 and 5,600.

The analysis of the total leucocyte count in this series thus serves to confirm the results of Vedder (1907) and of Stitt (1913). There is in the majority of cases of dengue a definite tendency to reduction in the number of circulating leucocytes, this becoming more marked and more constant as the disease progresses and frequently reaching its maximum after convalescence is well established. Our work adds to the results of the authors cited only the assurance of exact knowledge of the day of the disease, as the cases were all under observation before the onset. Ordinary spontaneous cases, on the contrary, as is shown elsewhere, frequently fail to report for treatment until the second or third day, and it is often impossible to fix the time of onset with accuracy. The degree of leucopenia shown in our cases is much less than that reported by others, notably Stitt (1913), who found 3,200 as the average value in one hundred cases. We are unable to explain this difference in results, but feel certain that our figures represent an unselected series of cases of varying degrees of severity.

In addition to this confirmation of the fact of a progressive leucopenia in dengue, we are also able to show that in this homogeneous series of cases, 25 per cent failed to show leucopenia at any stage of the disease. Moreover, a small series of counts taken within a few hours of the onset suggest that at that early stage of the disease an absence of leucopenia, or even a moderate increase in the number of circulating leucocytes, is not incompatible with the existence of dengue.

There were one hundred thirty counts in which it was possible to state the temperature at the time the blood was taken for examination. These cases are tabulated in Table 30.

This tabulation fails to suggest any regular correlation between the temperature at the moment and the volume of the leucocyte count. The four counts taken when the temperature happened to be above 103° are all below average, but the table taken as a whole fails to show any correlation. This is what would naturally be expected from the relation previously shown between the white blood count and the duration of the disease,

TABLE 30.—*Relation of white blood count to temperature at time of count.*

White blood count.	Sub-normal.	98.0–98.9	99.0–99.9	100.0–100.9	101.0–101.9	102.0–102.9	103.0–103.9	104.0–104.9	Total.
3,000 to 3,999	0	1	1	0	1	0	0	0	3
4,000 to 4,999	0	7	10	0	3	0	2	2	24
5,000 to 5,999	5	16	10	15	4	3	0	0	53
6,000 to 6,999	0	7	8	9	7	1	0	0	32
7,000 to 7,999	1	1	2	4	4	0	0	0	12
8,000 to 8,999	0	4	0	1	1	0	0	0	6
Total	6	36	31	29	20	4	2	2	130
Mean	5,883	5,592	5,500	6,190	6,150	5,750	4,500	4,500	------

in as much as many if not most of the counts made with the lower temperature ranges were taken late in the disease or in convalescence. This correlation failing, an attempt was made to ascertain whether or not there was any relation between the severity of the attack, as indicated by the highest temperature recorded during its course, and the degree of leucopenia. Of the forty-eight transmission cases studied, two were excluded from this comparison; one on account of the existence of a septic complication which raised the count, and the other on account of the loss of his clinical record. The tabulation of this relation is presented in Table 31.

TABLE 31.—*Relation of highest temperature observed to lowest white blood count.*

White blood count.	99.0–99.9	100.0–100.9	101.0–101.9	102.0–102.9	103.0–103.9	104.0–104.9	Total.	Mean.
3,000 to 3,999	0	1	0	2	0	0	3	101.8
4,000 to 4,999	1	4	6	1	1	3	16	101.7
5,000 to 5,999	0	3	9	7	1	0	20	101.8
6,000 to 6,999	0	0	1	4	0	0	5	102.3
7,000 to 7,999	0	0	1	0	0	0	1	101.5
8,000 to 8,999	0	0	1	0	0	0	1	101.5
Total	1	8	18	14	2	3	46	---------
Mean	4,500	4,750	5,720	5,429	5,000	4,500	------	---------

This tabulation fails to show any relationship between the severity of the case as indicated by the peak temperature observed and the degree of leucopenia. It suggests that the leucopenia and the degree of temperature are dependent upon different factors in the pathogenesis of the disease. There is equal failure of relationship between the severity of the disease, as measured by the duration of temperature, and the degree of leucopenia. This relationship is shown in Table 32.

TABLE 32.—*Relation of duration of temperature to lowest white blood count.*

White blood count.	Duration in days.							Total.	Mean.
	1	2	3	4	5	6	7		
3,000 to 3,999	0	1	0	1	1	0	0	3	4.20
4,000 to 4,999	1	4	5	4	1	0	1	16	3.25
5,000 to 5,999	1	5	3	4	2	4	1	20	4.35
6,000 to 6,999	0	0	1	0	4	0	0	5	5.1
7,000 to 7,999	0	0	1	0	0	0	0	1	4.5
8,000 to 8,999	0	1	0	0	0	0	0	1	2.5
Total	2	11	9	10	8	4	2	46	------
Mean	5,000	5,227	5,055	5,160	5,650	5,500	5,000	------	------

Failure to demonstrate any marked relationship between the temperature at the time of the count, the highest temperature observed during the attack, or the duration of the attack on the one hand, and the degree of leucopenia on the other, is perhaps surprising and, if confirmed by further studies, would tend strongly to indicate that the reaction of the leucocytes is to a great extent individual to the person attacked.

Differential count.—Vedder (1907) has shown that the percentage of polynuclear neutrophiles in dengue cases becomes progressively smaller as the disease progresses, accompanied by a corresponding increase in the proportion of lymphocytes. The differential counts made in the course of this study serve to confirm and to some extent amplify his results. Starting with the knowledge that the change in dengue is of the nature stated, we desired to obtain a single figure for each count which would represent this change in a comparative way. For this purpose the percentage of lymphocytes observed in a given count was divided by the corresponding percentage of polynuclear neutrophiles. The resulting decimal fraction may be termed a differential ratio, or in symbols $\frac{L}{N}$, if L represents the percentage of lymphocytes and N the percentage of neutrophiles. This ratio is merely a statistical tool to afford the opportunity of comparing the extent of the differential change with other variables observed in the study of the cases. If this ratio be calculated for each of the preliminary counts made on the experimental series, and the counts differing from the mean by more than 3P.E. be excluded, it is shown that the values of $\frac{L}{N}$ fall between 0.300 and 0.600 with a mean value slightly over

0.400 and a median value of 0.400. More than two-thirds of the values fall between 0.300 and 0.450. The curve of frequencies of this ratio, expressed in percentages of the total number of counts, is shown in fig. 19.

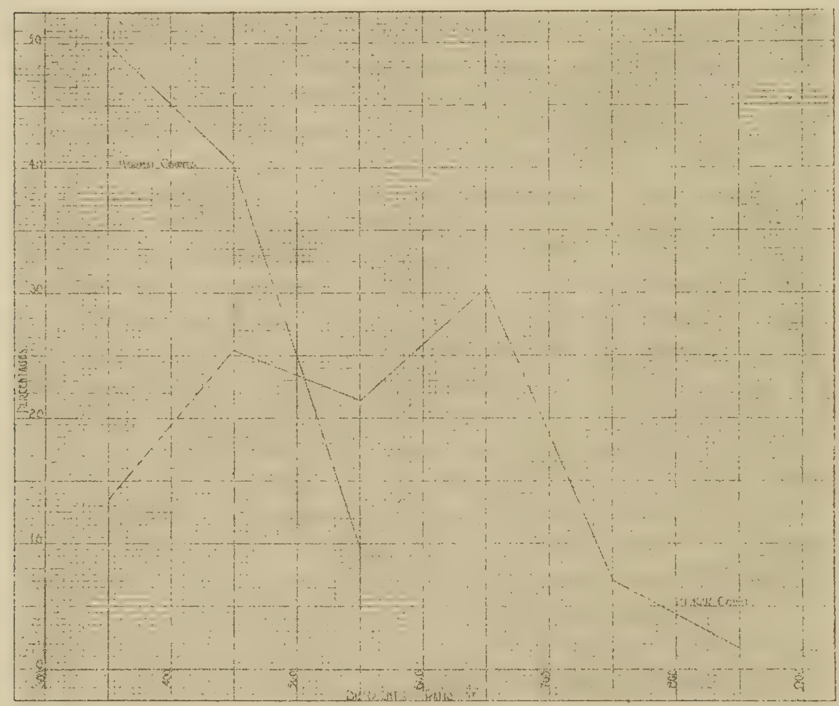

FIG. 19. Chart showing the percentage distribution of the differential ratio $\frac{L}{N}$ in normal and in dengue counts.

In calculating this ratio it was noticed that the higher values of $\frac{L}{N}$ tended to occur with the lower total counts. Accordingly, the figures for the two series were arranged in the correlation table, Table 33.

Table 33 at once suggests a definite correlation between the two sets of figures, the values of $\frac{L}{N}$ rising as the total count becomes lower. Accordingly, the coefficient of correlation was calculated and found to be — 0.52 with a probable error of 0.058. This coefficient in relation to the extent of the probable error is undoubtedly of statistical significance, and it may be safely stated accordingly that there is a distinct tendency for the values of $\frac{L}{N}$ to rise as the total number of circulating leucocytes be-

TABLE 33.—*Showing the relation between the differential ratio $\frac{L}{N}$ and the total leucocyte count. Normal individuals.*[a]

White blood count.	Differential ratio.					Total.	Mean.
	0.300–0.349	0.350–0.399	0.400–0.449	0.450–0.499	0.500–0.549		
5,000 to 5,999	0	0	0	0	3	3	0.525
6,000 to 6,999	1	4	6	2	2	15	0.425
7,000 to 7,999	3	6	8	3	2	22	0.414
8,000 to 8,999	2	13	7	0	0	22	0.386
9,000 to 9,999	5	2	3	0	0	10	0.365
Total	11	25	24	5	7	72	
Mean	8,500	8,020	7,792	7,100	6,625		

[a] Excluding all values differing from the means of the component distributions by more than 3P.E.

comes smaller; or, in other words, that with lower leucocyte counts there is a distinct tendency to a reduction in the neutrophile percentage and an increase in the proportion of lymphocytes. The curve plotted from the figures given in Table 33 is shown in fig. 20. The values for counts above 6,000 are seen to fall in a practically straight line. The deviation from this line of those for counts below 6,000 (only seven counts) may be accidental or may suggest that the relationship between the series is not linear, but is best represented by a curve. Accordingly,

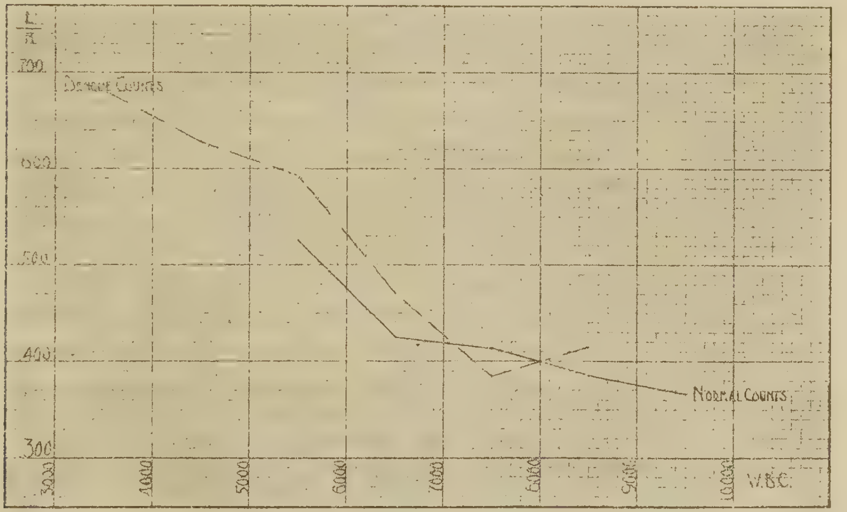

FIG. 20. Chart showing the relation of the differential ratio $\frac{L}{N}$ to the total leucocyte count in normal and in dengue counts.

Pearson's correlation ratio was calculated and this method gave a figure practically identical with the correlation coefficient given above, namely 0.534 ± 0.057. The application of Blakeman's formula gives a figure which indicates that the relationship between the total and the differential ratio may safely be regarded as linear within the normal range of leucocyte counts. However, should a larger number of normal counts show a similar deviation from the line of the value for the counts below 6,000, the suspicion might well be entertained that the line for still lower values of the white blood count would deviate from the straight line still more.

When we turn to the differential counts made on dengue cases subsequent to the onset, and exclude those whose total count varied more than 3P.E. from the mean of the corresponding daily distribution, we have one hundred twenty-six counts made at all stages of the disease. The results of these counts calculated as $\frac{L}{N}$ ratios are shown in Table 34.

Table 34.—*Relation of* $\frac{L}{N}$ *to total leucocytes in one hundred twenty-six dengue cases at all stages of the disease, subsequent to onset.*

White blood count.	Differential ratio.						Total.	Mean.
	0.300–0.399	0.400–0.499	0.500–0.599	0.600–0.699	0.700–0.799	0.800–0.899		
3,000 to 3,999	0	0	0	2	1	0	3	0.683
4,000 to 4,999	1	0	8	10	4	1	24	0.629
5,000 to 5,999	3	6	13	25	4	1	52	0.594
6,000 to 6,999	3	20	5	2	0	0	30	0.470
7,000 to 7,999	7	4	0	0	0	0	11	0.386
8,000 to 8,999	3	2	1	0	0	0	6	0.416
Total	17	32	27	39	9	2	126	0.549

The mean value of $\frac{L}{N}$ for all the dengue counts was, thus, 0.549, over three-quarters of the values falling above 0.450, whereas, as already stated, over two-thirds of the normal-count values fell below this figure. The median value for the dengue counts is 0.552. This distribution of values for dengue cases, expressed in percentages, is plotted in fig. 20, in comparison with the corresponding figures for the normal counts. While the difference between the two series is sufficiently striking, reference to fig. 19, which shows the corresponding curves for the total leucocyte counts, will convince one that the reduction in leucocytes is a more striking and characteristic feature of the dengue counts, as compared with the normals, than is the differential change. The flatter curve, with much greater average deviation from the mean of the series shown by the differential counts, may perhaps be evidence of a lesser degree of accuracy in the figures themselves. Most students of the subject will agree that, in general, differential counts, unless marked variations from the normal are shown, are valuable only when large numbers of cells, 500 or more, are counted. This usually proves impracticable in a busy service. The scatter of these values as compared with those of the total counts in the same series is the more remarkable when the relation between them and the total counts is considered.

The correlation coefficient calculated from Table 34 is —0.65 : 0.035, thus showing a negative correlation between the values even more marked than that of the normal cases. This relation is plotted in fig. 20 in comparison with the normals. It is seen on inspection of this chart that the relation here is definitely linear, but that the angle to the x axis taken by the line is somewhat greater than that characterizing the normal counts. The deviation of the lower normal counts is roughly parallel to the line of the dengue counts. This is in line with the suggestion made above, that the change in the differential relations may prove to proceed more rapidly in the case of values of the white blood counts below 6,000 than in those above that figure. The confirmation of this suspicion must await the study of a much larger series of cases.

Our figures have failed to show any confirmation of the statements made by other investigators as to the increase in the percentage of eosinophiles during the course of dengue. However, as the counts were made in the course of regular hospital routine, and not as a matter of research, we do not feel justified in contending that this late eosinophilia does not occur. The percentage of eosinophiles present in blood is so small that a large number of cells must be counted so that a reliable result may be obtained. Furthermore, Stitt (1907) based the diagnosis of dengue from allied conditions, notably influenza, largely on the results of differential counts. He found that in the earlier days of the disease there was an increase in the proportion of small lymphocytes; that, later, large lymphocytes predominated; and that these, in turn, were replaced by large mononuclear and transitional cells. Such change he regarded as following the developmental cycle of the mononuclear cell through youth, maturity, and old age and degeneration. It is believed that the later tendency in laboratory practice is to do away with the somewhat arbitrary distinction between large and small lymphocytes, while the large mononuclear and transitional cells are now believed to have an origin entirely distinct from the origin of the lymphocytes. These considerations led us to concentrate attention on the varying relations of the neutrophiles and the lymphocytes taken as a whole, no other interesting changes having been observed.

From the practical standpoint, the interesting thing shown by this analysis of the present figures appears to be that, in general, the differential count in normal cases and in dengue cases is a function of the total count. As the total count becomes smaller there is a diminution of the proportion of neutrophiles and an increase in that of the lymphocytes, the changes bearing a definite relation to the degree of change in the total count. The differences between the mean values of $\frac{L}{N}$ in normal and in dengue counts that fall within the range of white blood counts common to the two series is not significant and, therefore, so far as this study goes, the differential count would appear to be of little value in the diagnosis of dengue as compared with the estimation of the total number of leucocytes. It seems possible that analysis of the differential relations in a much larger series of known normal individuals, together with comparison with counts above as well as below normal, might bring out interesting relations and perhaps add to the definiteness of our interpretation of differential counts.

Other symptoms.—The tendency to disproportionate slowness of the pulse, as compared to the elevation of temperature, has already been mentioned. This symptom was observed in the majority of our cases. Ordinary ward observations of pulse are probably not as satisfactory as those of temperature, as it often happens that the pulse is counted under conditions which cause temporary acceleration. This accounts for the great irregularity seen in some of the curves of pulse rates. The usual observation, however, was to note a distinct relative slowing of the pulse after the first day. With the onset of fever the pulse usually rose about as would be expected in any other infection. In cases of several days' duration it was the rule to find the pulse dropping to normal or below while the temperature remained elevated. This was particularly noticeable in the cases showing diphasic temperature curves; in these it was usual to note a failure of the pulse to rise at all with the secondary elevation of temperature. Pronounced bradycardia was frequent during the early days of convalescence.

The blood pressure was taken with onset in the majority of cases. The average systolic pressure was 110. The subjects were all young men, and in the Islands there is a distinct tendency to reduction of blood pressure, as is shown in the figures collected during the annual physical examinations of officers. This figure, therefore, does not represent a reduction of pressure with the onset of the disease, but does indicate that there is no tendency to increase of tension in the early days of dengue, such as is frequent in sthenic fevers.

Albuminuria of slight degree was observed in two of our cases, once accompanied by granular casts. The condition was transient in both. Neither jaundice nor hæmorrhage occurred in our cases.

In considering the symptoms described one is impressed with the large proportion that may reasonably be attributed to involvement of the central nervous system. Of these headache, postorbital pain, loss or perversion of the sense of taste, vertigo, and mental depression form a group characterizing a majority of the cases and very evidently of central origin. The initial rash with its evanescent character may easily also be of central nervous mechanism. Major Riley's observation of the relief of symptoms on lumbar puncture is in line with this idea. The other distinctive symptoms—fever, adenopathy, and the changes in the leucocyte count—are what we should expect to characterize a blood infection. Dengue, therefore, appears to be an infection of the blood stream with toxic symptoms, involving especially the central nervous system. This conclusion is confirmed by the description of the post-mortem pathology of the disease given above.

SUMMARY

Comparison of the symptomatology of our experimental cases with the description of the disease given by Major Riley and with the symptomatology of the parallel series of cases occurring naturally during the period of our experiments gives convincing evidence that the virus with which we were dealing was in fact that of dengue.

The exceptional opportunity for clinical study of a series of forty-eight cases of dengue has allowed us to make exact observations on several points of interest.

1. Individual cases of dengue vary greatly in severity of subjective symptoms, intensity and duration of fever, and almost all other clinical manifestations.

2. Certain cases in our series were so mild that, in the absence of confirmation of diagnosis by further transmission of the virus, their inclusion in the series might well appear questionable.

3. The curves showing the frequency of cases of varying duration and varying intensity of fever suggest that dengue cases may occur in which fever is absent. Three cases, classified as negative in our experimental series, but showing suspicious symptoms after inoculation, offer further suggestive evidence to the same effect. No such cases were confirmed by transmission experiments, but it is our belief that afebrile dengue may occur, even in totally unprotected individuals, and that it may be a considerable factor in the spread of the disease.

4. The onset of dengue appears to be less abrupt than is usually believed. Sixty-five per cent of our cases showed prodromes lasting from six to forty-eight hours before the initial rise of temperature.

5. Adenopathy is shown to be present in the vast majority of cases at onset, and should prove a valuable differential point in diagnosis. Loss or perversion of the sense of taste is also a valuable early symptom.

6. The shortest period of incubation noted in our series was four and a quarter days, the longest ten. The average period of incubation was slightly over six days. Periods much longer than ten days are shown to be extremely improbable.

7. The average maximum temperature reached during the course of an attack was just less than 102° F. The average duration was three and three-tenths days. Forty per cent of the cases had definite "saddle-back," or diphasic, fever curves; 29 per cent had curves suggestive of the diphasic type; the remaining 31 per cent had simple single-wave curves. The relative rarity of the diphasic curve in spontaneous cases is shown to be due to the fact that they come under observation too late for the early fever to be noted.

8. The leucocyte count is not a dependable early symptom, as counts taken within a few hours of onset show great variation and average about normal. With the progress of the disease the total leucocyte count is reduced, the mean for the first four days becoming progressively less. However, 25 per cent of our

cases failed to show a leucopenia at any time during the course of the disease.

9. The so-called "reversal" in the differential count, the reduction of the percentage of polynuclear neutrophiles and corresponding increase of lymphocytes, is shown to be a function of the total count, both in normal and in dengue cases. Dengue cases showing normal total counts do not show the differential change. The differential count of the white cells is thus seen to be of little diagnostic value in comparison with the total count.

10. The degree of leucopenia appears to be independent of the temperature at the time the count was made, and also of the severity of the disease as judged by the highest temperature reached during the attack, or by the duration of fever.

11. In only two cases, or 4 per cent, was it impossible to demonstrate an eruption at some period of the disease. The initial rash failed of discovery in seven cases. In half the cases, both rashes were observed, separate and distinct. In nine, the early rash persisted throughout. The type of rash bore no relation to the character or duration of the temperature curve. The terminal rash appeared as a rule at the end of the febrile period, but this rule is subject to considerable variation.

12. Toxic involvement of the nervous system is a prominent feature of the symptomatology of dengue.

IMMUNITY

With recovery from dengue the patient possesses a degree of immunity to the infection which is decidedly individual in character. Convalescence is evidence that the immunizing forces of the body have successfully performed their function of overcoming the invading disease-producing agents. The permanence of such changed reactivity of the body tissues and fluids to the dengue virus is variable, however, and the reason therefor is not apparent. In some persons the refractory period is habitually brief, and two, three, or even four attacks may succeed one another before a sufficiently potent and durable immunity has been built up to insure freedom from further severe attacks. Other persons suffer but one attack recognizable as such, notwithstanding subsequent exposure in epidemic seasons, and a few individuals, even from nonendemic areas, seem to be naturally immune to dengue. With regard to natural immunity, the possibility cannot be ruled out by the evidence at hand that some individuals, especially those permanent residents in areas of dengue endemicity who seem to be naturally immune, maintain their resistance only through mild, usually unrecognized attacks, beginning probably in infancy. It has been suggested that the relatively low degree of immunity following dengue results from the fact that the disease runs a short course and has no death rate. Not even analogy can be brought to the support of this hypothesis.

There is a vast difference in susceptibility to dengue infection between those persons (except infants and young children) coming to an area of endemicity from a dengue-free district and those who have always lived in dengue territory. Intermediate between these groups are natives of localities like some in the southern United States where dengue is not endemic but is periodically epidemic. Infants and young children do not, of course, show such differences, at least not to the same extent as do adults.

It was with a knowledge of the published statements concerning repeated attacks in the same individuals that we undertook to obtain definite information with regard to the permanence and solidity of the immunity which follows one attack of dengue and that resulting from more than one attack. We have been able also to investigate the immunity in natives of the Philippine Islands.

This research has been made in two ways. The first may be called epidemiological, or statistical, and was made through the collection and analysis of data pertaining to the incidence and recurrence of dengue among American troops stationed in Manila. The second was experimental, and was based upon the results of subcutaneous injections of dengue virus into volunteers. There were two groups of volunteers. The first consisted of individuals known to have had a previous attack of dengue at a definite date, and injections were made to ascertain whether or not they still possessed acquired immunity at the time of the injection. The second series of volunteers so injected were native Filipino soldiers, and the purpose was to obtain information with regard to their so-called natural immunity.

STATISTICAL

First epidemiological study.—The epidemiological survey of dengue among American troops stationed in Manila, discussed in another section of this report, permitted the hope that evidence could be obtained from the same data—with certain other figures from the original sources—concerning attack rate, relapses, and recurrences of dengue among the individuals of those same groups. In other words, we felt that a study of these groups might give us fairly accurate information upon the relative susceptibility and immunity of the average American soldier in Manila under natural conditions as distinguished from the more or less artificial conditions of the experimental study of immunity, then well under way. We tabulated the information available in such a way as to show, as far as possible, what we were seeking. The tables gave the initials, rank, and organization of each of the dengue patients who had suffered more than one attack in the years 1922, 1923, and 1924, the dates of initial attacks and of subsequent attacks, and the intervals between attacks.

The tabulation showed immediately that serious errors would be included if we made percentage deductions from it, for the reason that the groups were not homogeneous. In the first place, the regular tour of duty in the Philippine Islands is two years. A few men apply for and receive extension of time to three years; a few reënlist and stay longer. None of the individuals of these groups, however, were permanent residents of the Philippine Islands, and the great majority returned to the United States about two years after their arrival in the Philippine Department. Then, those who had their initial attack in 1922, and passed through the epidemic season of the two subsequent years, had more opportunity to become infected than did those who had their initial attack in 1924. Complicating the matter still further is the fact that some of those who had attacks in 1922 and 1923 left for home before the next dengue season. Furthermore, any attempt to obtain accurate estimates concerning the prevalence of dengue is complicated by the fact that mild attacks are never recorded; only in those who are ill enough to be sent to the hospital is the condition definitely diagnosed as dengue, and recurrences are a little more likely than initial attacks to be mild. We have in this a factor which reduces the absolute as well as the comparative accuracy of any such statistical study.

A suggestion does seem to have been obtained with regard to the great variation in the intervals between attacks; this depends partly on chance infection and partly also, we believe, on differences in the duration of the acquired immunity. We feel, therefore, that this first epidemiological study contains information from which the inference can be drawn that the degree of resistance acquired by an attack of dengue is exceedingly variable in character and diminishes to a point where it is no longer effective at a rate that bears no consistent relationship with any factor known to us.

Second epidemiological study.—After the first epidemiological study had been completed and its value and limitations ascertained, we did not feel satisfied that we had exhausted the possibilities of our resources. Accurate and adequate records are, of course, on file concerning all the official movements of every soldier in the department, but their total volume is such that searching through them to obtain accurate information concerning the average applicability of a single factor may be so monumental a task as to make the process practically impossible or unprofitable. It becomes necessary, therefore, so to plan such an investigation that a reasonable amount of time will be productive of results. We felt there should be a method, even under these circumstances, by which epidemiological evidence of immunological interest might be elicited from the records available, free from the errors that are due to the frequent shifting of military personnel—errors not entirely eliminated from the first study, as already noted. The following plan was finally evolved:

From the records of the Thirty-first Infantry we took the cases of a group of men, picked carefully to eliminate all whose assignment or transfer would complicate the problem of obtaining true statistical data concerning the experience with dengue of the average soldier coming to Manila from the United States. First, we selected only those who had their initial attack of dengue between July 1, 1922, and June 30, 1923. From this group all were then eliminated except those who were first attached to the organization on or after January 1, 1922, and the records of all those who went home earlier than December 31, 1923, were discarded. The period of observation ended December 31, 1924. All except a very few of these men had come from the United States on a recent transport. Before joining their companies, they were held as casuals for a few days, as a rule. It is interesting to note that several of them were infected during their period as casuals and came down with dengue within a day or two after being "picked up" on their company rosters. They could not have passed through an attack of dengue and recovered from it during this interval. We have secured, then, a group of men who had their initial attack within a definite year; they could not have been exposed to dengue for more than six months before and they remained in their organization for at least six months subsequent to their original attack. Each individual in the group was exposed to dengue through at least one more season of epidemicity. Furthermore, this group is so related to the entire organization to which it belongs that it is possible to make mathematical comparisons that possess a high degree of accuracy.

Reference to Table 14 shows that in this period the average strength of the Thirty-first Infantry was one thousand eighty-six. During the year concerned in this study the Thirty-first Infantry had five hundred sixty-two cases of dengue. We found complete and satisfactory records of all except twenty-eight; therefore, for comparative purposes we must reduce the average strength and the total number of cases by 5 per cent (twenty-eight being 5 per cent of 562), giving us one thousand thirty-two as the adjusted average strength, and five hundred thirty-four as the adjusted total cases.

Records were obtained of four hundred twenty-one initial attacks during the period; but, because of necessary eliminations resulting from assignments to the organization earlier than January 1, 1922, or for some other reason, only two hundred ninety-nine (71 per cent) of these could be accepted for our present purposes. Thus, 29 per cent of the total first attacks were discarded in order that the corresponding percentage could be calculated of those exposed who had first attacks during these months; the total strength is likewise reduced to 71 per cent, giving us seven hundred thirty-three. Therefore, of the seven hundred thirty-three individuals exposed to dengue in the year beginning July 1, 1922, there were two hundred ninety-nine first attacks; that is, 40 per cent of the exposed population had initial attacks of dengue in this period. Of the two hundred ninety-nine who had their first attack during this year, two hundred had no recurrences up to December 31, 1924.

A study of the attack rate and recurrences tabulated gives us the results shown in Table 35.

TABLE 35.—*Percentages of attack and recurrence in dengue.*

Patients exposed who had one attack	40
Patients attacked who had at least one recurrence	33
Patients who, having had two attacks, suffered subsequent attacks	14
Patients who, having had three attacks, suffered subsequent attacks	7

We believe these figures represent fairly accurately the experience with dengue of the average American soldier coming to Manila for a period of two years. On the basis of these results, and rounding the figures off, we may say that under military conditions in Manila, 40 per cent of newly arrived troops are likely to acquire dengue within about a year; 30 per cent of these may expect a recurrence; those who have had two attacks are more immune and only 15 per cent of them will have another; of the few who have had three attacks before their departure for home, only 7 per cent will be likely to experience another attack severe enough to be diagnosed as dengue. The percentage of recurrences would naturally be higher if the period of residence here were longer than two years. Table 36 shows that 50 per cent of those who had three and four attacks of dengue had been here longer than two years.

Detailed results of the second epidemiological study are given in Tables 36 and 37.

The exact dates of arrival (this means, at the company—a few days, as a rule, subsequent to arrival in Manila) and dates of attack (and in every case, the first was the initial attack of that individual) are given, in order that those interested may see the time of year in which the attack occurred. This makes it possible to check these tables with the corresponding part of the section on Epidemiology. The figures of greatest interest are those of the intervals elapsing between arrival and attack, between attacks, and between the last attack and departure.

In the case of the two hundred soldiers of the Thirty-first Infantry who had only one attack, we have thought it sufficient to condense the results to a summary of the intervals between arrival and attack and between attack and departure. This summary is given in Table 38.

TABLE 36.—*Dengue in Thirty-first Infantry, 1922, 1923, and 1924; soldiers who had three or four attacks.*

Soldier.					First attack.		Second attack.		Third attack.		Fourth attack.		Departure.		Total service in Philippine Department (Manila) in months.
Case No.	Initials of patient.	Rank.	Company.	Date of arrival.	Date.	Days since arrival.	Date.	Days since first attack.	Date.	Days since second attack.	Date.	Days since third attack.	Date.	Days since last attack.	
1	F.E.T.[a]	Cpl	B	Mar. 18, 1922	Sept. 3, 1922	169	Jan. 12, 1923	131	Feb. 27, 1923	46	Apr. 23, 1923	55	June 21, 1923	59	15
2	H.C.C.	Pvt	G	June 12, 1922	July 25, 1922	43	Feb. 28, 1923	218	June 3, 1923	95	Sept. 24, 1923	113	Aug. 29, 1924	345	27
3	J.S.	Pvt	F	Feb. 13, 1922	Oct. 31, 1922	260	Jan. 1, 1923	62	Feb. 11, 1923	41			Dec. 31, 1924	689	35
4	W.D.	Pfc	Hdqr	Mar. 16, 1922	Jan. 2, 1923	292	Mar. 29, 1923	86	May 23, 1923	55			Jan. 9, 1924	231	22
5	J.M.	Pvt	D	Mar. 18, 1922	Oct. 5, 1922	201	May 21, 1923	228	Aug. 23, 1923	94			June 7, 1924	289	27
6	T.S.	Pfc	B	do.	Sept. 25, 1922	191	May 4, 1923	221	July 26, 1924	449			Aug. 29, 1924	34	29
7	H.H.	Cpl	D	Apr. 11, 1922	Dec. 22, 1922	255	May 7, 1923	136	Mar. 1, 1924	299			Nov. 8, 1924	252	31
8	D.W.S.	Pvt	I	do.	May 3, 1923	387	May 17, 1923	14	Apr. 4, 1924	329			do.	218	31
9	J.D.	Pvt	B	May 5, 1922	Oct. 19, 1922	167	Aug. 25, 1923	310	Oct. 6, 1924	408			Dec. 31, 1924	86	32
10	McK.O.	Sgt	I	July 3, 1922	Dec. 7, 1922	157	Mar. 26, 1923	109	July 9, 1923	105			June 7, 1924	334	23
11	A.B.C.	Pvt	E	July 4, 1922	May 5, 1923	306	Dec. 27, 1923	236	May 22, 1924	147			do.	16	23
12	F.H.R.	Pvt	G	Aug. 14, 1922	Oct. 27, 1922	74	Aug. 7, 1923	284	Apr. 9, 1924	246			do.	59	22
13	L.J.K.	Pvt	I	Sept. 9, 1922	Sept. 28, 1922	19	Sept. 3, 1923	340	Oct. 29, 1924	422			Dec. 31, 1924	63	28
14	J.M.	Pvt	B	do.	Oct. 18, 1922	39	Jan. 12, 1923	86	July 31, 1923	200			Sept. 2, 1924	399	24
15	D.W.S.[a]	Pvt	L	Oct. 7, 1922	Oct. 10, 1922	3	Mar. 23, 1923	164	June 29, 1923	98			Nov. 4, 1923	128	13
16	J.C.	Pvt	F	Jan. 11, 1923	June 3, 1923	143	Sept. 23, 1923	112	May 14, 1924	234			Nov. 8, 1924	178	22

[a] Left department prior to December 31, 1923, and for that reason not included in the analysis.

TABLE 37.—*Dengue in Thirty-first Infantry, 1922, 1923, and 1924; soldiers who had two attacks.*

Soldier.					First attack.		Second attack.		Departure.		Total service in Philippine Department (Manila) in months.	Case No.
Case No.	Initials of soldier.	Rank.	Company.	Date of arrival.	Date.	Days since arrival.	Date.	Days since first attack.	Date.	Days since last attack.		
1	J.A.	Pvt	Hdqr	Jan. 7, 1922	Oct. 8, 1922	274	Feb. 27, 1923	142	May 6, 1924	434	28	1
2	S.K.F.[a]	Pvt	F	Jan. 12, 1922	Jan. 13, 1922	1	June 3, 1923	506	Jan. 9, 1924	220	24	2
3	M.E.L.	Pvt	B	Feb. 1, 1922	Aug. 4, 1922	184	Aug. 24, 1922	20	Sept. 2, 1924	740	31	3
4	E.B.	Pfc	E	Feb. 3, 1922	Oct. 7, 1922	246	May 11, 1923	216	Jan. 9, 1924	243	23	4
5	J.R.E.[a]	Pvt	H	do	Mar. 4, 1923	394	Aug. 8, 1923	157	Sept. 9, 1923	32	19	5
6	J.H., Jr.	Pvt	F	do	Oct. 31, 1922	270	Apr. 6, 1923	156	Jan. 9, 1924	278	23	6
7	T.B.L.	Cpl	G	do	Oct. 9, 1922	248	Nov. 1, 1922	23	do	434	23	7
8	J.M.	Pvt	K	do	Aug. 29, 1922	207	June 4, 1923	279	Sept. 2, 1924	456	31	8
9	F.T.[a]	Pvt	M	do	July 29, 1922	176	June 14, 1923	312	June 21, 1923	7	17	9
10	H.D.	Pvt	E	Feb. 13, 1922	June 9, 1923	481	Nov. 3, 1923	147	Dec. 31, 1924	424	35	10
11	F.H.[a]	Pvt	F	do	Oct. 7, 1922	236	Mar. 10, 1923	154	May 3, 1923	54	14	11
12	W.J.K.	Pvt	B	do	Oct. 6, 1922	235	Oct. 20, 1922	14	Jan. 9, 1924	446	23	12
13	C.J.K.[a]	Pvt	K	do	Aug. 9, 1922	177	Oct. 27, 1922	79	Apr. 10, 1923	164	14	13
14	F.M.[a]	Pvt	K	do	Oct. 10, 1922	239	Apr. 11, 1923	183	Oct. 24, 1923	196	20	14
15	G.W.M.	Pvt	L	do	Oct. 1, 1922	230	Apr. 25, 1923	206	Jan. 9, 1924	259	23	15
16	A.P.	Pvt	L	do	Oct. 3, 1922	232	May 9, 1923	208	do	245	23	16
17	W.S.	Pvt	I	do	Sept. 20, 1922	219	Oct. 2, 1923	377	Dec. 31, 1924	456	35	17
18	F.S.[a]	Pvt	K	do	Sept. 2, 1922	201	June 11, 1923	282	June 21, 1923	10	16	18
19	F.Y.[a]	Cpl	L	do	Apr. 22, 1922	68	Oct. 9, 1922	195	Jan. 9, 1924	457	23	19
20	J.J.P.[a]	Pvt	A	Feb. 17, 1922	June 8, 1922	111	Nov. 8, 1922	153	Apr. 6, 1923	149	14	20
21	V.R.[a]	Pvt	C	Mar. 10, 1922	Mar. 30, 1922	20	Sept. 12, 1922	166	Oct. 12, 1922	31	7	21
22	G.B.	Pvt	C	Mar. 16, 1922	Oct. 16, 1922	214	Apr. 25, 1923	191	Jan. 9, 1924	259	22	22
23	T.W.B.[a]	Cpl	B	do	Jan. 15, 1923	305	June 14, 1923	150	Oct. 24, 1923	132	19	23
24	W.D.[a]	Pvt	E	do	Oct. 19, 1922	217	Apr. 7, 1923	170	Oct. 29, 1923	205	19	24
25	R.W.G.	Pvt	F	do	Oct. 14, 1922	212	Nov. 15, 1922	32	Jan. 9, 1924	420	22	25
26	R.E.J.[a]	Pvt	C	do	July 23, 1922	129	Dec. 21, 1922	181	Apr. 10, 1923	110	13	26

27	R.O.	Pvt	E	do	Oct. 12, 1922	210	Dec. 8, 1922	57	Jan. 9, 1924	397	22	27
28	J.W.P.[a]	Pvt	K	do	Aug. 13, 1922	150	Mar. 25, 1923	224	Nov. 15, 1923	235	20	28
29	W.F.S.[a]	Pvt	K	do	June 28, 1922	104	Sept. 23, 1922	87	Nov. 7, 1922	45	8	29
30	L.L.W.[a]	Pvt	E	do	May 19, 1922	64	Aug. 9, 1922	82	Sept. 8, 1922	30	6	30
31	M.E.B.	Pvt	B	Mar. 18, 1922	Oct. 18, 1922	214	May 2, 1923	196	Oct. 24, 1924	541	31	31
32	W.B.[a]	Pvt	L	do	Oct. 1, 1922	197	May 17, 1923	228	Aug. 7, 1923	82	17	32
33	H.H.	Pvt	L	do	Sept. 4, 1922	175	May 10, 1923	248	Jan. 9, 1924	244	22	33
34	W.J.J.	Pfc	M	do	Oct. 14, 1922	210	July 12, 1923	271	do	181	22	34
35	A.M.[a]	Pvt	M	do	Oct. 9, 1922	205	June 27, 1923	261	Sept. 5, 1923	70	18	35
36	W.R.S.[a]	Pvt	E	Mar. 27, 1922	Sept. 21, 1922	178	Aug. 6, 1923	319	Oct. 24, 1923	79	19	36
37	M.E.B.	Cpl	M	Apr. 1, 1922	May 2, 1923	396	June 28, 1923	57	Jan. 4, 1924	190	21	37
38	L.F.[a]	Pvt	C	Apr. 11, 1922	Sept. 9, 1922	151	Oct. 24, 1922	45	July 10, 1923	259	15	38
39	J.E.H.	Pvt	E	do	Oct. 10, 1922	182	June 22, 1923	255	Mar. 24, 1924	276	23	39
40	H.G.M.	Pvt	Hdqr	do	Sept. 9, 1922	151	Apr. 10, 1923	213	do	349	23	40
41	J.M.V.	Pvt	I	do	Sept. 24, 1922	166	Apr. 5, 1923	193	do	354	23	41
42	G.F.W.	Pvt	C	do	Oct. 2, 1922	174	Apr. 21, 1923	201	Nov. 15, 1924	574	31	42
43	P.J.G.	Pvt	M	do	Sept. 21, 1922	163	June 21, 1923	273	Mar. 24, 1924	277	23	43
44	R.T.B.	Pvt	I	Apr. 21, 1922	Aug. 12, 1922	113	May 9, 1923	270	do	320	23	44
45	B.B.	Pvt	M	May 5, 1922	Sept. 26, 1922	144	June 10, 1923	257	do	288	23	45
46	L.V.D.	Pvt	B	do	Oct. 10, 1922	158	Sept. 8, 1923	333	do	198	23	46
47	H.H.[a]	Pvt	G	do	Nov. 10, 1922	189	Dec. 18, 1922	38	Oct. 29, 1923	315	18	47
48	N.B.H.	Pvt	L	do	Sept. 12, 1922	130	Apr. 29, 1923	229	Mar. 24, 1924	330	23	48
49	W.C.T.	Pvt	I	do	Feb. 13, 1923	283	June 18, 1923	125	Nov. 15, 1924	516	30	49
50	C.A.J.[a]	Sgt	B	June 10, 1922	Oct. 1, 1922	113	Oct. 11, 1922	10	Jan. 15, 1923	96	7	50
51	J.E.G.	Pvt	A	June 12, 1922	Sept. 15, 1922	95	Oct. 31, 1922	46	June 7, 1924	585	24	51
52	E.H.	Pvt	B	do	Oct. 8, 1922	118	July 13, 1923	278	do	329	24	52
53	J.J.	Pvt	B	do	Sept. 30, 1922	110	Dec. 20, 1922	81	do	535	24	53
54	M.P.K.	Pvt	C	do	July 31, 1922	49	June 9, 1923	312	Mar. 24, 1924	289	21	54
55	C.S.	Pvt	K	do	Jan. 3, 1923	205	June 28, 1923	176	June 7, 1924	345	24	55
56	E.S.	Pvt	B	do	Sept. 23, 1922	103	Feb. 22, 1923	152	do	471	24	56
57	M.H.W.	Pvt	L	do	Sept. 26, 1922	106	Oct. 9, 1922	13	Mar. 24, 1924	532	21	57
58	E.L.T.	Sgt	L	July 3, 1922	Sept. 30, 1922	89	Dec. 15, 1922	76	June 7, 1924	540	23	58
59	J.D.B.	Pvt	L	July 4, 1922	Sept. 6, 1922	64	Oct. 9, 1922	33	do	604	23	59
60	G.C.	Pvt	C	do	Nov. 2, 1922	121	Apr. 27, 1923	176	do	437	23	60

[a] Left department prior to December 31, 1923, and for that reason not included in the analysis.

TABLE 37.—*Dengue in Thirty-first Infantry, 1922, 1923, and 1924; soldiers who had two attacks*—Continued.

Soldier.					First attack.		Second attack		Departure.		Total service in Philippine Department (Manila) in months.	Case No.
Case No.	Initial of soldier.	Rank.	Company.	Date of arrival.	Date.	Days since arrival.	Date.	Days since first attack.	Date.	Days since last attack.		
61	W.H.D.	Pvt	D	July 4, 1922	July 25, 1922	21	Apr. 26, 1923	275	May 6, 1924	376	22	61
62	J.L.G.[a]	Pvt	F	do	Oct. 30, 1922	118	July 2, 1923	245	Aug. 9, 1923	38	13	62
63	E.D.G.	Pvt	E	do	Aug. 5, 1922	32	Oct. 7, 1922	63	June 7, 1924	609	23	63
64	C.C.K.	Pvt	E	do	Mar. 15, 1923	254	June 12, 1923	89	do	361	23	64
65	G.S.M.[a]	Pvt	C	do	Sept. 22, 1922	80	May 19, 1923	239	Sept. 11, 1923	115	14	65
66	M.R.M.[a]	Pvt	H	do	Sept. 29, 1922	87	Oct. 10, 1922	11	Jan. 15, 1923	97	6	66
67	H.A.R.	Pvt	I	do	July 25, 1922	21	Nov. 19, 1923	482	June 7, 1924	201	23	67
68	E.H.S.[a]	Pvt	K	do	Sept. 19, 1922	77	Oct. 9, 1922	20	Mar. 24, 1923	166	9	68
69	L.E.N.	Pvt	G	Aug. 14, 1922	do	36	May 24, 1923	247	Feb. 6, 1924	258	18	69
70	E.E.B.[a]	Pvt	A	Sept. 6, 1922	Oct. 14, 1922	38	Jan. 24, 1923	102	Apr. 4, 1923	70	7	70
71	D.C.	Pvt	F	do	Nov. 3, 1922	53	May 26, 1923	204	Aug. 29, 1924	461	24	71
72	A.D.	Pvt	M	do	Sept. 20, 1922	14	Aug. 8, 1923	322	do	387	24	72
73	S.G.	Pvt	M	do	Sept. 22, 1922	16	July 2, 1924	649	do	58	24	73
74	H.E.G.	Pvt	M	do	Sept. 29, 1922	23	May 16, 1924	595	do	105	24	74
75	M.H.G.	Pvt	M	do	Sept. 21, 1922	15	June 20, 1923	272	do	436	24	75
76	E.J.G.	Pvt	M	do	Oct. 22, 1922	46	June 25, 1923	246	do	431	24	76
77	C.E.G.	Cpl	I	do	June 17, 1923	284	Aug. 9, 1924	419	do	20	24	77
78	J.H.	Pvt	C	do	Sept. 23, 1922	17	May 19, 1923	238	do	468	24	78
79	F.D.	Pvt	D	Sept. 9, 1922	Sept. 30, 1922	21	Apr. 28, 1923	210	do	489	24	79
80	T.H.	Pvt	M	do	Sept. 21, 1922	12	June 16, 1923	268	do	440	24	80
81	J.G.L.	Pvt	G	do	Dec. 27, 1922	109	May 26, 1923	150	Sept. 3, 1924	466	24	81
82	A.A.L.	Pvt	K	do	Sept. 23, 1922	14	June 12, 1923	262	Aug. 29, 1924	444	24	82
83	C.P.M.[a]	Pvt	G	do	Apr. 3, 1923	206	July 6, 1923	94	Dec. 20, 1923	167	15	83
84	J.P.	Pvt	K	do	Sept. 22, 1922	13	Aug. 18, 1923	330	Aug. 29, 1924	377	24	84
85	O.C.T.	Pvt	I	do	Sept. 19, 1922	10	Apr. 27, 1923	220	do	490	24	85

86	E.A.V.[a]	Pvt	I	do	Oct. 13, 1922	34	Feb. 25, 1923	135	Mar. 13, 1923	16	6	86
87	E.W.	Pvt	K	do	Sept. 21, 1922	12	June 12, 1923	264	Aug. 29, 1924	444	24	87
88	R.J.M.	Pvt	Hdqr	Sept. 16, 1922	Oct. 18, 1922	32	Apr. 20, 1923	184	do	497	23	88
89	J.B	Pvt	B	Oct. 7, 1922	Apr. 20, 1923	195	May 31, 1924	407	do	90	23	89
90	C.V.C.[a]	Pvt	B	do	Dec. 19, 1922	73	June 12, 1923	175	Nov. 2, 1923	143	13	90
91	J.P.C	Pvt	K	do	Oct. 27, 1922	20	Apr. 9, 1924	530	Aug. 29, 1924	142	23	91
92	M.P.F	Pvt	L	do	Oct. 9, 1922	2	Nov. 8, 1922	30	do	660	23	92
93	H.M.H	Pvt	L	do	Oct. 10, 1922	3	May 21, 1923	223	do	466	23	93
94	R.V.H.[a]	Pvt	D	do	Nov. 13, 1922	27	May 16, 1923	184	July 10, 1923	144	9	94
95	W.Q.J	Pvt	L	do	Oct. 9, 1922	2	do	219	Aug. 29, 1924	471	23	95
96	F.S	Pvt	L	do	Oct. 10, 1922	3	Apr. 11, 1923	183	do	506	23	96
97	H.A.R	Pvt	Hdqr	Oct. 16, 1922	Apr. 29, 1923	195	Apr. 15, 1924	352	do	136	22	97
98	O.A.H.[a]	Pvt	E	Nov. 14, 1922	Mar. 15, 1923	121	June 16, 1923	93	Dec. 28, 1923	195	13	98
99	W.A.R	Pvt	F	do	May 24, 1923	191	Apr. 23, 1924	335	Aug. 29, 1924	128	21	99
100	R.G	Pvt	B	Dec. 5, 1922	Jan. 11, 1923	37	May 23, 1923	132	do	464	21	100
101	C.C	Pvt	F	Jan. 11, 1923	Feb. 16, 1923	36	Oct. 29, 1924	621	Nov. 8, 1924	10	22	101
102	J.D.	Pvt	F	do	Jan. 30, 1923	19	Aug. 28, 1924	576	do	72	22	102
103	T.B.F	Pvt	L	do	May 17, 1923	126	June 18, 1923	32	Dec. 31, 1924	562	24	103
104	S.E.J	Pvt	A	do	Feb. 20, 1923	40	June 1, 1923	101	Nov. 8, 1924	526	22	104
105	R.E.J	Pvt	C	do	May 8, 1923	117	July 25, 1923	78	do	472	22	105
106	P.M.L	Pvt	L	do	May 10, 1923	119	June 6, 1923	27	do	521	22	106
107	C.L.O.	Pvt	M	do	June 2, 1923	142	May 9, 1924	342	do	183	22	107
108	M.F.R.	Pvt	B	do	Feb. 19, 1923	39	Aug. 24, 1923	186	do	442	22	108
109	L.F.F.	Pvt	G	Mar. 13, 1923	June 29, 1923	108	Aug. 5, 1924	403	Dec. 31, 1924	148	22	109
110	J.E.G.	Pvt	E	do	June 12, 1923	91	May 6, 1924	329	do	239	22	110
111	G.D.M.	Pvt	F	do	Apr. 24, 1923	42	July 5, 1923	72	do	545	22	111
112	C.J.C.	Pvt	I	Apr. 1, 1923	May 30, 1923	59	July 19, 1923	50	do	532	21	112
113	U.G.D.	Pvt	M	do	May 10, 1923	39	May 20, 1924	376	do	226	21	113
114	E.G.	Pvt	I	do	May 12, 1923	41	Aug. 26, 1924	472	do	127	21	114
115	H.K.	Pvt	M	do	Apr. 25, 1923	24	June 16, 1923	52	Dec. 7, 1924	540	20	115
116	J.E.M.	Pvt	L	do	May 28, 1923	57	Aug. 28, 1923	92	Dec. 31, 1924	491	21	116
117	N.C.	Pvt	B	June 24, 1923	June 27, 1923	3	Apr. 17, 1924	295	do	259	18	117

[a] Left department prior to December 31, 1923, and for that reason not included in the analysis.

TABLE 38.—*Dengue in Thirty-first Infantry, 1922, 1923, and 1924; soldiers who had one attack without recurrence.*

1. Interval between arrival and attack and number of soldiers who had dengue after the interval.

Interval between arrival and attack.	Soldiers having dengue after the interval.
Days.	
1 to 7	3
7 to 10	1
10 to 15	5
15 to 20	4
20 to 25	5
25 to 30	6
30 to 35	3
35 to 40	5
40 to 45	5
45 to 50	4
50 to 60	4
60 to 70	2
70 to 80	2
80 to 90	3
90 to 100	9
100 to 120	19
120 to 140	10
140 to 160	16
160 to 180	8
180 to 200	19
200 to 225	16
225 to 250	10
250 to 300	16
300 to 350	6
350 to 400	8
400 to 450	4
450 to 500	6
500 to 525	1
Total	200

2. Interval between attack and departure and numbers attacked.

Interval between attack and departure.	Soldiers attacked.
Days.	
180 to 200	1
200 to 250	6
250 to 300	9
300 to 350	3
350 to 400	8
400 to 450	16
450 to 500	25
500 to 550	23

TABLE 38.—*Dengue in Thirty-first Infantry, 1922, 1923, and 1924; soldiers who had one attack without recurrence*—Continued.

Interval between attack and departure	Soldiers attacked.
Days.	
550 to 600	25
600 to 650	26
650 to 700	24
700 to 750	11
750 to 800	7
800 to 850	15
850 to 875	1
Total	200

Table 39, which concerns those who had two attacks, is a summarization similar to that of Table 38.

TABLE 39.—*Dengue in Thirty-first Infantry, 1922, 1923, and 1924; soldiers who had one recurrence.*

1. Interval between arrival and first attack and numbers attacked after the interval.

Interval between arrival and attack.	Soldiers having dengue after the interval.
Days.	
1 to 10	5
10 to 15	6
15 to 20	4
20 to 25	6
25 to 30	0
30 to 35	2
35 to 40	5
40 to 45	3
45 to 50	2
50 to 60	3
60 to 70	1
70 to 80	0
80 to 90	1
90 to 100	2
100 to 120	9
120 to 140	3
140 to 160	4
160 to 180	4
180 to 200	5
200 to 250	13
250 to 300	5
300 to 350	0
375	1
475	1
Total	85

TABLE 39.—*Dengue in Thirty-first Infantry, 1922, 1923, and 1924; soldiers who had one recurrence*—Continued.

2. Interval between first and second attacks, and numbers attacked.

Interval between arrival and attack.	Soldiers having dengue after the interval.
Days.	
13 to 15	2
15 to 20	0
20 to 25	2
25 to 30	1
30 to 35	4
35 to 40	0
40 to 45	0
45 to 50	1
50 to 60	4
60 to 70	1
70 to 80	3
80 to 90	2
90 to 100	1
100 to 120	1
120 to 140	2
140 to 160	5
160 to 180	2
180 to 200	6
200 to 225	10
225 to 250	5
250 to 275	9
275 to 300	4
300 to 350	7
350 to 400	3
400 to 450	3
450 to 500	2
500 to 550	1
550 to 600	2
600 to 650	2
Total	85

3. Interval between second attack and departure.

Interval between attack and departure.	Soldiers attacked.
Days.	
10	1
20	1
55 to 60	1
70 to 75	1
90 to 100	1
100 to 120	1
120 to 140	3
140 to 160	2

TABLE 39.—*Dengue in Thirty-first Infantry, 1922, 1923, and 1924; soldiers who had one recurrence*—Continued.

Internal between attack and departure. Days.	Soldiers attacked.
160 to 180	0
180 to 200	4
200 to 225	2
225 to 250	4
250 to 275	6
275 to 300	3
300 to 350	5
350 to 400	6
400 to 450	12
450 to 500	14
500 to 550	10
550 to 600	4
600 to 650	2
650 to 700	1
700 to 750	1
Total	**85**

The indication that recovery from dengue results in a period of immunity is shown clearly in the columns giving the interval between attack and departure. In several cases this was brief, but in the majority it stretched over at least one more epidemic season.

The intervals between final attack and departure are in general shorter among those who had two or more attacks than in those who had but one. Relatively few of those who had but one attack went home before the next dengue season; thirty-four of the two hundred were here through two subsequent seasons.

From Table 38 there seems to be no relation between the length of the interval from arrival to first attack and between the periods elapsing from one subsequent attack to the next. In other words, there is no indication that initial high susceptibility—if a short interval before the first attack indicates high susceptibility—means a greater or less tendency to acquire immunity of durable length. The two soldiers who had four attacks both show a lack of any appreciable lengthening of their well periods between their successive attacks.

This second epidemiological study makes more emphatic the conclusion drawn from the first; that is, that recovery from an attack of dengue results in a degree of acquired immunity which is exceedingly variable in its effective duration. We believe this point can be considered definitely established.

It adds definite information upon the percentage of recurrences that may be expected in the average soldier in Manila during a tour of service lasting two or three years.

At least 40 per cent of military personnel will have attacks severe enough to demand hospitalization.

After a delay of two hundred to three hundred days, in the great majority of cases, 30 per cent of those attacked will have severe recurrences. A few will have even third and fourth attacks, violent enough to require hospitalization, before their departure.

Nothing is known concerning the relative numbers of mild attacks which the soldiers have and never report. Common talk indicates that they are numerous.

The diagnoses in these cases were all made by the chiefs of the Medical Service at Sternberg General Hospital. We are certain the question will be asked by some readers: "Were not some, at least, of these illnesses which were called dengue—especially, some of the recurrences—as a matter of fact, malaria paroxysms?" Ashburn and Craig (1907, page 133), in their discussion of immunity and susceptibility, say:

> The correctness of reports in cases in which attacks have occurred a month apart we very much doubt. We had about six patients sent back to us after such periods, supposed to be suffering from second attacks, but in no case was it so. The "second attack" was usually a malaria paroxysm.

Malaria could scarcely have played an important rôle as a cause of the attacks which we have accepted as dengue. The troops concerned had come from the United States directly to Manila, where relatively few persons become infected with malaria, and had not seen service in parts of the Islands where malaria is prevalent. It is true that some of them probably had malaria at home and a few may have been carriers; individuals may have made visits to malarious districts here. If these possibilities actually were operative in causing the attacks we have accepted as dengue, it is strange that the malaria should have manifested itself always in just this way, and with parasites so rare in the circulating blood as to result in failure to find them in the cases in which microscopic examinations were made.

The treatment for dengue does not include the administration of quinine and, while malaria may indeed manifest itself as an occasional febrile attack of brief duration, this occurs chiefly in places where blood examinations are not made. It seems impossible that there should be such intervals as those noted above, in so many men, between attacks so brief—due to malaria and with no quinine given during or subsequent to such illnesses.

The medical chiefs at the hospital made the diagnosis in each case without knowledge as to whether it was a primary attack or a recurrence, and the type of study made to establish diagnosis is clearly set forth in the clinical section of this work (Clinical Aspects, pages 170 to 211). In order to compare the symptoms exhibited by naturally acquired cases with the symptoms of those produced experimentally, "as a comparison and control a similar tabulation of the symptomatology shown by a series of twenty-four naturally acquired cases, occurring consecutively during the period covered by our work, is also shown."

In order to obtain exact information upon the occurrence of malaria and its prevalence in the Thirty-first Infantry during the years 1922, 1923, and 1924, we have carefully searched the records at Sternberg General Hospital. The primary object was to find out in how many of the first and subsequent attacks of dengue blood examination for malaria had actually been made. It soon became evident that other diagnoses than "dengue" were interesting from the standpoint of diagnosis. It seemed that such a noncommittal record as "Febricula" might throw a little light on the prevalence of mild and atypical dengue—or malaria. For this reason we noted all instances in which the diagnosis made was "Fever, type undetermined," "Febricula," or "Migraine." We added to the list also all the cases diagnosed "Malaria." The information obtained is presented in Table 40.

TABLE 40.—*Malaria in Thirty-first Infantry in 1922, 1923, and 1924.*

Dengue, one attack only:	
Cases (patients)	283
No examination for malaria	232
Examined and negative—	51
Only one examination	40
Two examinations	10
Three examinations	1

TABLE 40.—*Malaria in Thirty-first Infantry in 1922, 1923, and 1924*—Ctd.

Dengue, recurrences:	
Patients—	133
No examinations for malaria	82
At least one examination	51
One examination only	40
Two examinations	10
Three examinations	1
These 133 patients had attacks	284
Charts not found	4
No examination	219
Examined and negative	51

	1922	1923	1924
Fever, type undetermined:			
Cases	1	21	14
Not examined for malaria	1	9	0
Examined for malaria and negative	0	12	14
One case on dengue list.			
Febricula:			
Cases	4	1	0
Not examined for malaria	1	1	0
Examined for malaria and negative	3	0	0
Two on dengue list.			
Two not on dengue list but had malaria.			
Migraine:			
Cases	0	4	0
Not examined for malaria	0	4	0
Three on dengue list.			
Malaria:			
Cases	20	1	2
Blood examination positive	20	1	2

The most striking of all these figures are those relating to positive diagnoses for malaria; these were always based on blood examination. The one positive case in 1923 and the two in 1924 make paludism practically negligible during the period when the great majority of the dengue relapses occurred. Among our dengue patients were five who had previously had malaria during the period of our study. Even if we set aside the negative examinations for malaria made later, at the time the dengue diagnosis was made, and agree that the so-called dengue was really malaria (we do not, however) no material change would be made in our figures. The dates of the malaria and dengue attacks in these five soldiers are shown in Table 41; the following symbols are used in this Table as well as in Tables 42 and 43:

+, Blood examined and found positive for malaria parasites.
—, Blood not examined for parasites.
=, Blood examined and found negative; the figure indicates the number of examinations that were made.

TABLE 41.—*Diagnoses for malaria and dengue in five patients.*

Name.	Disease.	Date.	Blood examination.	Remarks.
Grenuk, Wasil	Malaria	May 2, 1922	+	
Do		June 16, 1922	+	
Do	Dengue	Oct. 14, 1922	—	Dengue rash. No quinine. Recovered.
Lamb, Chester A.	Malaria	June 23, 1922	+	
Do	Dengue	Apr. 24, 1923	=	Fever curve suggests dengue. Recovered without quinine.
McArthur, Andrew	Malaria	July 26, 1922	+	
Do	Dengue	Oct. 9, 1922	=	Dengue rash; 5,600 leucocytes; other symptoms indicate dengue. No quinine.
		June 27, 1923	—	Symptoms of dengue. No quinine.
Egan, James P.	Malaria	Sept. 8, 1922	+	
Do	Dengue	Mar. 4, 1923	=	Typical severe dengue. No quinine.
		Aug. 8, 1923	—	Clinically, dengue. No quinine.
Masek, John	Malaria	Nov. 16, 1922	+	
Do	Dengue	May 27, 1923	=	Clinical record does not seem to exclude malaria. Quinine administered before last examination of blood for parasites.

Two men had malaria and later febricula; neither was ever admitted to the hospital for dengue (see Table 42).

TABLE 42.—*Diagnoses for malaria and febricula in two patients.*

Name.	Disease.	Date.	Blood examination.
Brighton, Wm.	Malaria	Apr. 20, 1922	+
Do	Febricula	July 5, 1922	=
Turner, Darius M.	Malaria	June 19, 1922	+
Do	Febricula	Sept. 5, 1922	=

Interesting combinations of dengue and other diagnoses are seen in Table 43.

TABLE 43.—*Diagnoses for dengue and other ailments combined, in four patients.*

Name.	Disease.	Date.	Blood examination.
Center, Wayne T	Febricula	July 17, 1922	=
Do	Dengue	Nov. 2, 1922	—
Do	Migraine	Mar. 23, 1923	—
Lee, Marion E	Dengue	Aug. 4, 1922	=
		Aug. 24, 1922	=
Do	Febricula	Sept. 1, 1923	—
Alders, Gerald A	Dengue	Oct. 3, 1922	—
Do	Migraine	Feb. 16, 1923	—
Scully, Wm	Dengue	Dec. 2, 1922	—
Do	Fever, type undetermined	Oct. 24, 1923	—

The lists include the names of all those in our dengue tables who had at other times febricula, fever (type undetermined), migraine, or malaria.

The records of Sternberg General Hospital show clearly, we believe, that malarial infection was not so prevalent in the Thirty-first Infantry as to interfere seriously with the accuracy of the diagnoses for dengue during the years 1922, 1923, and 1924.

EXPERIMENTAL

The tables, together with the deductions to be drawn from the figures presented, give information of great interest; but they give definite answers to only a few of the important questions concerning immunity to dengue. In addition to the fact that we possess no record of mild attacks of dengue which may have occurred in our various groups, the factor of infection is by no means a certain one; it cannot be claimed that all individuals in the groups were equally exposed to the bites of *Aëdes* mosquitoes, nor that the mosquitoes themselves were infected uniformly. It will be remembered that Ashburn and Craig (1907) found one man who never was bitten by mosquitoes at all; he might have been considered naturally immune to dengue, had these investigators not injected him with virus blood and found him susceptible.

Taken by itself, then, we feel that only the general conclusions which constitute the opening paragraph of the epidemiological part of our study on immunity in dengue should be drawn from that section. Fortunately, we are not compelled to leave the discussion in a condition so incomplete. In the course of this research we have had a unique opportunity to obtain information experimentally upon the question of immunity to dengue, and the circumstances have been such that no reservations are required in discussing the results.

Several of the individuals who came down with dengue in the experimental ward consented to be injected, at a subsequent date, with blood drawn from persons in the early stages of typical attacks of dengue produced experimentally. A few of those who volunteered for the immunity test had had definite attacks, contracted otherwise than in the experimental ward—spontaneous attacks.

In addition to the tests for acquired immunity made upon persons who had had previous attacks at definitely known dates, a series of injections of virus blood were made into native Filipinos. Only three of the Filipinos remembered having had an attack of dengue, and their recollection was extremely vague—we felt that the innate politeness of the East was the chief cause of the attacks.

In Table 44 are given the data concerning the experimental tests for immunity made by the subcutaneous injection of virus blood into persons, the dates of whose previous attacks were accurately known.

The infecting virus.—Ashburn and Craig (1907) found that the virus of dengue is present in the circulating blood; Cleland and Bradley (1916) have shown that the virus-carrying blood retains its infectivity for several days if it is kept under proper conditions, and the work of this board has shown that the period of greatest potency, in so far as the infection of mosquitoes is concerned, is short, possibly two days, beginning a few hours before the appearance of symptoms.

All the patients from whom the virus blood was taken for these experiments had been ill less than forty-eight hours (Byam A–48, Maloy A–62, Driscoll A–72, Foos A–86, Byrd A–91, and Duncan A–101 were in the first day; Kibbe A–28 and White A–54 were in the second day), and all had given three clear negative Wassermann tests since entering the experimental ward. In no case was there an interval greater than four hours between the collection and the injection of the blood.

At least one person in each group of those injected with the blood of Kibbe, Byam, White, and Maloy came down with dengue,

TABLE 44.—*Tests for acquired immunity to dengue.*

Experiment No.	Volunteer.	Days since initial symptoms of last attack.	Virus.		Result.		Duration of attack in days.	
			Donor.	Day of dengue.	Positive (+) or negative (—).	Incubation period in (days).	Previous.	Resulting.
I–1	Prock B–2	53	Kibbe A–28	Second	+	3.5	4	2
I–2	Reed B–1	53	do	do	—	--------	4	-----
I–3	Richards B–3	53	do	do	—	--------	5	-----
I–4	Sparling[a]	98	do	do	+	5	4	3
I–5	Alexander A–20	40	Byam A–48	First	—	--------	3	-----
I–6	Eldert[a]	55	do	do	+	5	5	3
I–7	Ables A–18	48	do	do	—	--------	3	-----
I–8	Ross[a]	74	do	do	—	--------	7	-----
I–9	A.W.S.[a]	121	do	do	+	11	5	2
I–10	Marine[a]	50	White A–54	Second	—	--------	6	-----
I–11	Clark, H. G.[a]	90	do	do	—	--------	8	-----
I–12	Berkowitz[a]	86	do	do	+	7	6	4
I–13	Butler[a b]	91	Maloy A–62	First	—	--------	4	-----
I–14	Reiser[c d]	--------	do	do	+	--------	-----	-----
I–15	Judy[c e]	--------	do	do	--------	--------	-----	-----
I–16	Cain A–34	68	Driscoll A–72	do	—	--------	7	-----
I–17	Caron A–35	67	do	do	—	--------	5	-----
I–18	Carter A–26[e f]	--------	do	do	--------	--------	-----	-----
I–19	Dembowski A–52	46	do	do	—	--------	2	-----
I–20	Kibbe A–28	69	do	do	—	--------	5	-----
I–21	Watkins A–40	60	do	do	—	--------	4	-----
I–22	White A–54	32	do	do	—	--------	2	-----
I–55	Butler[a]	[b]145–54	Duncan A–101	do	—	--------	([b])	-----
I–56	Eldert[a g]	116–61	Byrd A–91	do	+	5	3	2(?)

[a] Spontaneous infection; that is, not produced experimentally.

[b] Butler I–13 tested first after ninety-four days, found immune. Tested again as I–55, found to be still immune: total, one hundred forty-eight days.

[c] Not included in summary and discussion.

[d] No definite history of previous attack.

[e] See B–14.

[f] Under observation only five days.

[g] See discussion of this case in text.

thus demonstrating positively the presence of potent virus in the inoculum. There were no positive results in the groups injected with the blood of Driscoll and Foos, but both these individuals were in the first day of a typical attack produced by the bites of infected mosquitoes. Within an hour or two of withdrawing the blood from Driscoll and Foos, *Aëdes* mosquitoes were permitted to take blood from them. The mosquitoes of lot 808, which were allowed to bite Driscoll on this day, subsequently produced dengue in Hockett A–90; those that bit Foos were lot 811 and subsequently produced dengue in McAllister A–96. We feel, therefore, that there can be no reasonable doubt as to the presence of active virus in the blood of Driscoll and of Foos. Byrd and Duncan were in the first day of typical attacks of dengue produced experimentally by the bites of infected mosquitoes, and it is scarcely possible that their circulating blood did not contain the virus. There was no mosquito control, as in the cases of Driscoll and Foos, and there were no positive indications of infection in the groups injected, unless we consider the slight and brief rise in temperature shown by some of the native volunteers a positive result, and unless we consider the result in Eldert I-56 really an attack of dengue.

From each of the donors of the virus about 10 cubic centimeters of blood were drawn from a vein, at the bend of the elbow, into about 0.5 cubic centimeter of sterile 1 per cent sodium citrate solution in physiological saline. Injections were made subcutaneously in the region of the insertion of the deltoid muscle; the amount injected was uniformly 0.5 cubic centimeter. In preparation for the injection, the skin was painted with iodine and the excess of iodine was washed off with acetone.

Result of test for acquired immunity.—Twenty-three persons giving a positive history of dengue were injected with virus blood, and two (Butler and Eldert) were injected a second time after intervals of fifty-four and sixty-one days, respectively. Butler was found to be still immune, while Eldert was susceptible; one (Carter) could be observed for only five days subsequent to the inoculation. Judy was possibly naturally immune; he was given a number in our table when we thought a slight indisposition was the beginning of an attack; now, we do not know, but some of us are inclined to believe he had such a reaction to the virus as so-called naturally immune natives of endemic areas exhibit. This point is discussed below and in the section on Clinical Aspects (pages 170 to 211). Reiser I-14 is included in our table, but subsequent examination of the facts in his case revealed that he had had no definite previous attack of dengue. The reason for including his name is that his positive attack, following injection of the blood from Maloy, constitutes a test of the infectiveness of the blood injected into Butler.

Therefore, we can include in the test group nineteen volunteers, five of whom, about 26.3 per cent, came down with the disease. If we include the second injections made into Butler and Eldert, we have twenty-one volunteers with six positive results, 28.6 per cent. These percentages are strikingly close to the percentages of recurrence found in the epidemiological study discussed above—30 per cent of those who had had but one previous attack. The intervals between the first day of the previous attack and the injection of virus-carrying blood were, in the positive cases, 53, 55, 86, 98, and 121 days. The longest interval, between the previous attack and the later injection, in which the man was found to be immune was ninety days (Clark, H. G.).

In this work, then, no individual injected earlier than fifty-three days (one volunteer was susceptible in so short a time) after his previous attack exhibited susceptibility to dengue infection; one individual, injected ninety days after his previous attack, was found to be immune.

The results for the second injection of Eldert I–56 deserve some discussion. Eldert was admitted first to Sternberg General Hospital with a "spontaneous" attack of dengue, which lasted five days: fifty-five days later he was injected, as I–6, with the blood of Byam A–46. After an interval of five days, he came down with what was considered a typical attack of dengue. Sixty-one days subsequent to the beginning of this attack, he was again injected, this time as I–56, and with the blood of Byrd A–91. The injection was made on February 12, 1925; on February 16, he complained of slight headache and post orbital soreness and pain, but had no rise in temperature. On the next day the symptoms were worse, but his temperature did not rise above 99° F.; he complained of vertigo, poor appetite, and lack of taste-sense, and claimed that he, himself, had noticed a slight mottling on his chest and upper abdomen. Personal contact with this volunteer and his familiarity with the symptoms of dengue made us hesitate to include this as a positive attack without reservations.

It is most interesting in this connection to note that on April 22, 1925, Eldert was admitted to the dengue ward of the hospital complaining that the day before he had had a slight headache, which became severe during the afternoon, and was accompanied by pains in the eyes and dizziness. In the evening he had a chill. On the morning of admission he had, in addition, pains in the back, no vomiting, but loss of appetite. According to the clinical record he had a macular eruption (mottled) with acne on the shoulders. On the evening of admission his temperature was 102° F. It reached normal on the afternoon of the 24th, and he was returned to duty on the 28th. His leucocytes were normal in number, and no malaria parasites were found in his blood. This attack, if dengue, would probably lend support to the diagnosis of dengue in the second experimental attack, and would indicate that Eldert is one of the individuals whose immunity to dengue is exceedingly evanescent.

Of more than passing interest is the case of Ross. He was tested for immunity to dengue as I–8 on December 8, 1924, and found to be insusceptible to the infection. On April 14, 1925, two hundred one days following his initial attack and one hundred twenty-seven days following the immunity test, he was admitted to Sternberg General Hospital, suffering with a typical attack of dengue, accompanied by a measleslike rash. His temperature on admission was 102° F., and increased to 103.8° F. the same evening. It did not reach normal until the 22d and he was returned to duty on the 24th. From the standpoint of acquired immunity, this occurrence, a naturally acquired attack of dengue, is important and may serve as the starting point for interesting observations upon the possibility of vaccinal immunity to dengue. He was immune to infection seventy-four days after his initial attack, but came down with a naturally acquired attack of dengue one hundred twenty-seven days after the injection of infective virus blood; it would seem reasonable to look for increased immunity, possibly of a very solid type, as the result of this volunteer's experience with the infection. The fact that there was no such result leads to the question as to whether or not a practical method for securing active immunity to dengue can be worked out.

A point of immunological interest is revealed by comparison of the duration of the initial attack with that of the attack following the injection of the virus. It will be noted from Table 42 that, in each instance, the experimentally produced attack was shorter than the previous one. The average duration of initial attacks was 4.8 days (excluding Eldert I–56); the average duration of the attacks resulting from the injection of the virus was only 2.8. It may be argued that there is a vast difference between infection through mosquito bites and infection through the injection of a large amount of virus-carrying blood. We see, however, that Prock B–2 received his initial infection through the injection of virus blood, and his first attack lasted four days, whereas his second lasted only two days. We believe this difference in the duration of the attack may be considered an indication that, although the volunteer's refractory state was not great enough to prevent infection altogether, he did have an appreciable degree of acquired immunity. The duration of the initial attack seems to have no essential relation to the degree of immunity resulting from it. This is indicated by the fact that the average duration of the disease in those who were found to be immune was 4.6 days—practically the same as in those who were found to be susceptible, 4.8 days.

Tests for natural (?) immunity.—It has been recorded by several observers that natives of areas where dengue is endemic possess a high degree of immunity. Whether this is in the nature of a natural immunity or whether it results from repeated attacks so mild as to be unrecognized, is a question for which there is at present no answer. We are inclined to believe the immunity is acquired. It seems possible that the earliest infection might have occurred in infancy and that subsequent infections have come along with sufficient frequency to fall within the period when there has been just enough residual immunity to make the attack brief in duration and mild in degree, although not enough to prevent it altogether. The actual tests of immunity we have been able to make have had for their purpose, first of all, to prove the assertion that natives of dengue endemic areas actually are immune.

The virus used has already been described. Three groups of Filipino soldiers volunteered for the tests; the groups were injected, respectively, with the blood of Foos A–86 (January 27, 1925), Byrd A–91 (February 12, 1925), and Duncan A–101 (February 27, 1925). The donors were in the first day of their attack. The blood, soon after its collection, was carried to the hospital at Fort William McKinley and injected subcutaneously, always within four hours of its collection; the dose was 0.5 cubic centimeter. The injected soldiers were not excused from their regular duties, except when admitted to the hospital. Their temperatures were taken at about 7 a. m., and at about 5 p. m., the first record having been made on the day of injection; temperatures of the third group were also taken immediately before the injection. Three of the volunteers believed they had had dengue before; the others had no recollection of any attack. In order that the results of these injections can be readily studied, the recorded temperatures following the injections are given for each of the volunteers in Table 45.

It will be noted that several of those injected had some rise in temperature on the day of injection, two on the first day thereafter, and two on the third. Considering the fact that the average incubation period in our mosquito transmission cases has been 4.25 days, these increases in temperature can scarcely have any significance in so far as dengue is concerned. It may be suggested that they are anaphylactic in nature, and this is an attractive thought; but more work devoted to this particular point must be done before we can express any opinion about it.

We have so frequently seen in the experiment ward at Sternberg General Hospital temperatures of 99.2° among volunteers during their quarantine period as to be certain that only temperatures above this point can be considered fever.

To obtain further information upon the normal temperature of the average Filipino soldier we had this same group report at the Fort William McKinley Hospital every day for two weeks, morning and evening. This was done about four months subsequent to the injection of virus. One or two of the men had been discharged or detached and not all were able to report every time. The figures obtained seem sufficient for the purpose. They are shown in Table 46.

It will be seen (Table 46) that Silvano had fever on the seventh and eighth days. This was due to an injection of typhoid vaccine, as noted on the chart.

Paule had fever on the morning of the ninth day and on the morning of the twelfth. So far, we have not been able to demonstrate malaria parasites in the blood of this man; he states that he never had malaria, but the interval between the two rises in temperature would make one look persistently for a malarial element in this case. Asistin, Laspiñas, de la Rosa, and Abad also had slight rises in temperature for which no definite explanation can be found. The fact that so many of them registered 99.4° F. makes it clear that a temperature at this level is not uncommon, and we cannot bring any rise not definitely greater than this into our discussion of possible reactions to the dengue virus.

Eliminating from our discussion, then, those soldiers who had slight rises in temperature on the third day after injection or before (and at no other time), and all those who had temperatures not above 99.4°, there remain several cases which seem to be worth detailed analysis.

TABLE 45.—*Tests for natural (?) immunity, Filipino troops, immediately subsequent to injection.*

Volunteer.					Virus.				Temperatures on day of injection and for fourteen days subsequently.							
No.	Name.	Age.	Home town and province.	Previous attack remembered.	Donor.	Day of disease.	Day injected.		First.		Second.		Third.		Fourth.	
		Yrs.					*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*
I-23	Torres	25	Aringay, La Union	None.	Foos A-86	First	------	98.6	99.0	98.6	98.4	98.4	98.8	(a)	98.6	97.8
I-24	Rojas	25	Palompon, Leyte	do	do	do	------	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
I-25	Alegrado	27	Lobo, Batangas	do	do	do	------	99.4	99.0	98.2	98.0	98.0	98.2	(a)	98.4	98.2
I-26	Asistin	22	Vigan, Ilocos Sur	do	do	do	------	98.6	98.4	98.4	98.6	97.2	98.2	(a)	98.2	98.2
I-27	Puri	22	Alitagtag, Batangas	do	do	do	------	98.4	98.2	98.4	97.0	98.4	98.0	(a)	98.2	98.4
I-28	Mendoza	22	Liau, Batangas	do	do	do	------	98.4	97.2	98.4	97.4	98.0	96.8	98.4	98.6	97.0
I-29	Buss	23	Angeles, Pampanga	do	do	do	------	98.6	98.6	98.4	97.6	98.6	97.6	98.0	96.2	98.0
I-30	Ferrer	33	Urbistondo, Pangasinan	do	do	do	------	98.6	98.6	98.4	(a)	98.0	98.8	(a)	98.2	(a)
I-31	Villamayor	31	Mauban, Tayabas	do	do	do	------	98.3	98.2	99.2	98.0	98.2	98.2	(a)	97.2	97.6
I-32	Laspiñas	24	Bais, Oriental Negros	do	do	do	------	97.2	98.6	98.2	99.0	98.2	97.0	98.4	98.2	98.6
I 33	Paule	31	Lobas, Pampanga	do	do	do	------	99.2	98.8	98.2	98.2	97.6	97.4	98.0	98.2	98.4
I-34	Bacani	33	Guagua, Pampanga	do	do	do	------	98.7	98.4	98.0	98.4	98.0	97.2	(a)	97.4	98.0
I-35	Canlas	45	Macabebe, Pampanga	do	do	do	------	98.6	97.8	98.3	98.6	98.2	96.8	(a)	97.2	98.0
I-36	Cerrano	44	Apalit, Pampanga	do	do	do	------	99.2	97.2	(a)	(a)	(a)	(a)	(a)	(a)	(a)
I-37	Macasait	26	(b)	do	do	do	------	98.6	97.4	98.6	98.2	98.2	98.6	98.4	98.6	98.4
I-38	de la Rosa	38	Santa Maria, Ilocos Sur	do	Byrd A-91	do	------	99.0	98.6	98.4	98.0	98.0	98.6	99.0	98.8	98.0
I-39	David	19	Concepcion, Tarlac	Yes.	do	do	------	97.0	97.6	96.0	97.0	98.6	97.2	97.8	98.4	96.8
I-40	de Castro	21	Alaminos, Pangasinan	None.	do	do	------	99.0	98.6	98.2	98.0	98.2	98.0	98.6	98.0	97.8
I-41	Soriano	44	Macabebe, Pampanga	Yes.	do	do	------	98.4	98.2	98.7	97.4	98.2	98.6	98.6	98.0	98.6
I-42	Torio	25	Binalonan, Pangasinan	do	do	do	------	98.8	98.2	97.6	98.2	98.6	97.2	98.6	97.0	98.6
I-43	Abad	24	Cabanatuan, Nueva Ecija	None.	do	do	------	98.4	98.2	98.8	98.2	98.0	97.8	98.6	98.2	99.8
I-44	Silvano	24	Tulusa, Leyte	do	do	do	------	98.2	98.4	98.6	98.0	98.4	98.2	98.4	98.6	99.0
I-45	Nicolas	36	Laoag, Ilocos Norte	do	do	do	------	98.7	97.0	98.6	98.0	(a)	98.6	98.6	99.2	98.7
I-46	Lepalam	22	Bago, Cebu	do	do	do	------	99.0	98.4	98.2	98.1	98.6	98.2	98.4	98.2	98.6

I-47	Mallillin	48	Zolana, Cagayan	do	do	do	------	98.0	98.2	97.8	98.0	(a)	97.4	98.0	97.0	98.4
I-48	Nicdao	31	Carizara, Leyte	do	Duncan A-101	do	98.0	99.0	97.2	98.4	96.8	98.4	97.5	99.4	98.4	98.7
I-49	Ordunia	24	Buenavista, Iloilo	do	do	do	99.4	99.6	99.2	98.2	98.8	99.0	98.6	99.0	98.8	99.0
I-50	Trillanes	20	Tigbauan, Iloilo	do	do	do	98.4	99.6	97.6	98.2	98.0	99.0	98.3	98.6	98.0	101.0
I-51	Cawaring	29	Sumaraga, Samar	do	do	do	98.6	99.2	99.0	99.0	(a)	(a)	98.8	99.0	98.6	98.6
I-52	Daza	26	Dingle, Iloilo	do	do	do	98.4	98.0	98.0	98.6	98.4	98.4	98.0	(a)	96.4	98.4
I-53	Baltazar	(b)	(b)	do	do	do	97.5	98.0	96.6	97.2	97.8	99.0	98.3	99.4	98.0	98.0
I-54	Acosta	(b)	(b)	do	do	do	99.2	100.2	98.0	97.6	98.4	98.6	98.8	98.6	98.6	98.0

No.	Temperatures on day of injection and for fourteen days subsequently.																			
	Fifth.		Sixth.		Seventh.		Eighth.		Ninth.		Tenth.		Eleventh.		Twelfth.		Thirteenth.		Fourteenth.	
	a.m.	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*	*a.m.*	*p.m.*
I-23	97.0	98.6	98.0	97.8	99.2	98.6	97.4	98.0	98.4	98.4	97.4	98.2	98.0	98.2	98.6	98.8	97.8	98.4	(a)	(a)
I-24	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
I-25	98.6	98.0	97.0	99.0	98.4	98.6	98.2	98.2	98.0	98.4	98.4	98.6	98.0	98.6	97.2	(a)	97.2	98.4	(a)	(a)
I-26	99.0	98.6	97.8	99.0	98.0	98.2	98.2	98.6	98.6	98.0	98.2	98.6	98.4	98.6	98.0	98.0	97.8	97.6	(a)	(a)
I-27	99.2	98.2	98.2	98.2	98.2	97.4	98.4	98.6	96.8	97.2	98.2	98.6	98.0	97.8	96.8	99.2	(a)	(a)	(a)	(a)
I-28	98.6	98.0	97.8	98.8	98.2	98.2	96.8	98.4	97.8	97.8	98.4	97.2	98.0	98.8	97.2	97.8	98.2	98.6	(a)	(a)
I-29	97.0	97.8	98.4	98.6	98.0	98.6	98.4	98.0	98.2	98.6	98.6	98.8	97.4	98.0	98.4	97.4	(a)	(a)	(a)	(a)
I-30	97.2	98.6	98.2	(a)	99.4	98.2	98.4	98.2	98.6	98.4	97.2	98.6	98.0	98.6	98.2	97.6	97.8	98.2	(a)	(a)
I-31	98.2	98.0	98.2	97.6	97.2	97.2	98.4	97.4	98.0	98.2	97.6	98.4	98.4	98.0	98.6	98.2	97.8	98.4	(a)	(a)
I-32	98.4	99.0	98.2	98.2	97.6	98.4	98.0	98.4	98.6	98.4	97.2	98.2	98.4	(a)	97.8	98.6	(a)	(a)	(a)	(a)
I-33	97.2	98.0	97.0	98.4	98.2	97.4	98.4	98.2	98.2	98.6	98.2	98.0	97.2	98.6	98.2	97.6	99.2	97.2	(a)	(a)
I-34	98.0	98.0	97.0	98.4	98.2	98.0	98.2	96.8	96.8	98.2	97.6	98.0	98.6	98.6	98.4	98.4	(a)	(a)	(a)	(a)
I-35	(a)	99.2	98.0	98.4	97.0	98.2	98.6	96.8	98.2	98.6	98.0	98.6	97.4	97.0	98.6	99.0	98.0	98.4	(a)	(a)
I-36	98.2	(a)	96.2	98.2	97.0	98.4	98.6	98.0	98.2	98.6	98.2	98.6	98.2	97.4	97.8	98.2	(a)	(a)	(a)	(a)
I-37	98.6	98.0	98.0	99.0	98.4	98.4	98.6	98.2	98.4	98.6	98.4	98.6	98.4	98.2	98.4	96.8	97.8	98.6	(a)	(a)
I-38	98.6	98.4	97.4	98.0	98.6	98.0	98.6	98.6	98.6	98.2	98.0	98.2	98.2	98.4	98.4	98.6	97.8	(a)	(a)	(a)
I-39	98.3	98.0	97.4	97.4	97.4	98.0	97.0	98.2	97.0	98.4	97.0	98.0	97.0	98.6	98.8	98.8	98.4	(a)	(a)	(a)
I-40	98.4	98.6	98.2	99.0	98.4	98.0	98.3	99.8	98.4	98.6	98.0	98.4	98.4	97.8	(a)	(a)	(a)	(a)	(a)	(a)

[a] Did not report to have temperature taken.

[b] Not known; records not available in Board offices.

TABLE 45.—*Tests for natural (?) immunity, Filipino troops, immediately subsequent to injection*—Continued.

No.	Temperatures on day of injection and for fourteen days subsequently.																			
	Fifth.		Sixth.		Seventh.		Eighth.		Ninth.		Tenth.		Eleventh.		Twelfth.		Thirteenth.		Fourteenth.	
	a. m.	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
I-41	98.6	98.6	(a)	98.4	(a)	98.4	98.0	100.0	97.2	100.8	97.6	98.6	98.0	98.0	97.2	(a)	(a)	(a)	(a)	(a)
I-42	97.0	98.0	99.4	99.2	98.6	99.6	98.4	98.4	97.6	98.0	96.2	98.6	97.4	97.8	97.5	98.6	98.0	(a)	(a)	(a)
I-43	97.0	97.2	97.0	98.0	98.0	101.0	99.0	98.7	97.5	99.2	98.0	98.0	97.0	98.2	(a)	(a)	(a)	(a)	(a)	(a)
I-44	97.0	96.8	99.2	101.2	98.0	98.6	98.3	99.2	97.6	98.4	98.0	98.2	96.2	98.6	(a)	(a)	(a)	(a)	(a)	(a)
I-45	99.0	98.0	98.2	98.6	98.0	99.2	98.0	99.2	98.3	98.0	98.0	98.4	97.0	98.8	(a)	(a)	(a)	(a)	(a)	(a)
I-46	97.4	96.8	98.0	98.6	97.0	98.6	98.0	99.0	98.2	98.2	98.2	98.6	97.6	98.8	97.8	98.6	98.0	(a)	(a)	(a)
I-47	98.0	98.4	98.2	98.2	98.0	98.0	98.0	98.6	98.0	97.6	98.2	97.8	97.6	98.0	97.8	98.2	97.8	98.2	(a)	(a)
I-48	97.6	98.3	98.4	98.4	98.0	98.4	98.6	98.6	97.0	98.3	98.6	98.4	(a)	98.0	98.4	(a)	98.0	(a)	98.4	(a)
I-49	98.8	98.0	98.4	98.2	98.8	99.5	99.8	100.0	98.7	99.2	97.0	97.0	97.0	99.4	101.4	101.0	(a)	99.8	98.0	99.4
I-50	99.0	99.3	98.6	99.0	98.6	98.0	98.0	98.4	97.8	98.0	97.0	97.8	97.4	97.0	99.3	98.2	98.8	98.4	98.6	98.2
I-51	98.8	98.0	98.6	99.0	98.0	99.0	98.6	(a)	98.8	98.2	98.2	98.4	98.4	(a)	99.0	98.4	98.6	(a)	98.2	(a)
I-52	97.8	98.0	98.0	98.0	98.6	98.0	98.4	98.0	98.0	98.0	97.0	98.2	97.8	98.0	(a)	98.0	97.8	(a)	(a)	(a)
I-53	(a)	98.0	98.2	98.8	98.6	98.0	97.7	(a)	98.0	98.4	97.0	98.2	97.6	98.0	97.8	(a)	98.0	98.4	98.0	(a)
I-54	98.6	99.0	98.4	98.0	98.6	98.6	98.6	98.0	97.6	99.1	(a)	99.0	98.4	98.6	(a)	(a)	(a)	(a)	(a)	(a)

[a] Did not report to have temperature taken.

[b] Not known; records not available in Board offices.

Before taking up this matter, however, let us refer to the case of Ordunia I-49. We see that he had some fever on the day of his injection, again on the seventh and eighth days, and on the twelfth, thirteenth, and fourteenth. The injection was made into the members of his group on February 27, 1925. As a result of his afternoon temperature on March 7, he was admitted to the Station Hospital, Fort William McKinley. He was studied carefully by Major Hoffman for some clinical indication of dengue, but no such diagnosis could be made. He had had malaria in 1923, but examination of his blood at this time revealed no parasites. No parasites could be found in any other member of his group. Blood examination showed 17,800 leucocytes, with 4 per cent eosinophiles, and examination of his fæces revealed hookworm ova. He was given treatment for ankylostomiasis and discharged from the hospital March 16, 1925, with his fæces free of ova. Of course, the slight degree of fever this soldier showed on the eighth day after his injection might have been due to an abortive attack of dengue; but it seems a little more likely that it was due to something else, especially since he had at the same time a mild degree of leucocytosis.

Private Abad stated that on the days when he had fever he had permitted himself to become constipated; that he was not sick at all. Accepting this as a plausible explanation, we shall not include him among those who possibly reacted to the dengue virus.

Private Trillanes was also admitted to the hospital. He was injected on February 27, and admitted on March 4, the fourth day following the injection, as a result of his fever. Most careful examination was made and daily observations were continued to detect a symptom which would seem to be pathognomonic of dengue, but none could be found. In common with the other native soldiers in these groups, who had fever subsequent to injection, Trillanes claimed that his only symptom was headache. He had no pains in his limbs and the headache was not associated with his eyes, there were no enlarged glands, and there was no rash detectable at any time. In his blood no malaria parasites could be found. The single point in the record of his case that might be considered as definitely suggestive of dengue is the small number of leucocytes in his circulating blood—6,350; polymorphonuclears, 56 per cent; mononuclears, 32 per cent;

TABLE 46.—*Temperatures of Filipino volunteers four months subsequent to injection of dengue virus.*

Volunteer.		June 10, 1925. First.		June 11, 1925. Second.		June 12, 1925. Third.		June 13, 1925. Fourth.		June 14, 1925. Fifth.		June 15, 1925. Sixth.		June 16, 1925. Seventh.	
No.	Name.	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
I-23	Torres	97.6	97.0	97.2	98.6	98.5	98.6	96.8	98.6	98.2	98.6	98.2	99.0	98.6	98.6
I-24	Rojas	(a)	(a)	(a)	(a)	98.5	97.8	(a)	(a)	(a)	98.4	(a)	(a)	97.8	98.0
I-25	Alegrado	97.8	98.0	97.2	99.0	97.4	98.4	97.6	98.4	98.6	97.4	98.0	98.2	97.8	98.6
I-26	Asistin	98.8	98.4	98.6	97.6	98.2	98.8	98.4	99.0	98.0	(a)	98.4	98.2	98.2	98.0
I-27	Puri	98.6	97.8	97.8	98.2	98.5	98.2	97.6	98.0	98.2	97.8	97.0	97.8	98.0	98.2
I-28	Mendoza	98.2	98.4	98.0	97.0	98.2	98.2	97.8	97.8	98.2	97.4	97.2	98.6	98.2	98.2
I-29	Buss	98.0	98.6	98.0	97.6	97.8	98.2	98.2	98.0	97.0	98.2	97.4	98.0	97.6	(a)
I-30	Ferrer	98.6	98.0	98.2	97.2	(a)	98.0	98.6	98.4	98.4	98.0	97.8	97.2	98.0	98.4
I-31	Villamayor	98.4	98.6	98.0	98.2	98.4	(a)	98.4	98.0	98.2	(a)	97.8	98.6	99.0	98.4
I-32	Laspiñas	98.2	(a)	(a)	97.6	98.7	98.2	96.2	98.0	98.2	(a)	97.4	98.4	98.0	98.4
I-33	Paule	98.4	98.4	98.2	97.8	97.4	98.4	98.0	98.0	98.2	98.4	98.4	97.8	97.2	98.6
I-34	Bacani	98.0	98.4	98.4	97.8	97.6	97.8	97.8	97.6	98.0	(a)	97.0	(a)	98.4	98.4
I-35	Canlas	98.2	98.6	98.0	98.3	99.0	98.4	98.2	98.6	96.8	97.6	99.0	98.4	98.0	98.2
I-36	Cerrano	(a)	(a)	(a)	98.6	98.6	(a)	98.0	98.4	97.8	(a)	97.6	98.0	97.6	(a)
I-37	Macasait[b]	---	---	---	---	---	---	---	---	---	---	---	---	---	---
I-38	De la Rosa	98.2	98.6	98.4	98.5	98.7	99.0	98.2	98.4	98.2	98.6	98.6	98.6	98.6	98.8
I-39	David	(a)	(a)	(a)	99.0	98.4	98.2	98.2	98.0	(a)	98.4	98.4	98.2	98.0	98.8
I-40	De Castro	98.0	(a)	97.8	(a)	97.8	97.8	97.8	(a)	(a)	(a)	98.0	(a)	98.0	98.0
I-41	Soriano	(a)	(a)	(a)	99.2	98.4	98.6	98.0	98.4	97.8	98.6	98.2	99.0	97.2	99.0
I-42	Torio	(a)	98.6	98.8	98.6	98.8	97.8	97.6	99.0	98.0	98.6	98.2	98.8	96.8	98.0
I-43	Abad	98.6	98.4	97.8	97.6	98.4	98.6	98.2	97.0	96.8	97.2	97.8	98.3	98.6	97.8
I-44	Silvano	99.0	98.6	98.4	97.6	98.2	98.4	97.6	98.0	97.8	97.6	97.4	97.6	98.0	98.0
I-45	Nicolas	98.4	98.6	98.2	98.4	99.0	99.0	98.4	98.4	97.2	98.0	98.8	97.8	97.8	[c]100.0
I-46	Lepalam	98.8	98.0	98.2	98.4	98.9	98.8	98.0	99.0	98.0	98.8	98.6	98.0	98.0	98.6
I-47	Mallillin	98.4	98.4	98.2	98.0	96.4	97.2	97.2	98.0	98.0	98.0	98.2	98.0	96.8	98.0

I-48	Nicdao	98.0	98.0	98.6	98.6	98.6	98.2	98.8	(a)	97.6	98.0	97.4	98.2	98.0	98.2
I-49	Ordunia	98.8	98.6	97.2	98.4	98.8	98.2	97.2	98.6	97.2	(a)	(a)	(a)	(a)	(a)
I-50	Trillanes	(a)	98.6	97.4	97.6	(a)	97.4	(a)	98.0	98.0	98.2	(a)	98.4	96.8	98.6
I-51	Cawaring	98.4	98.4	98.0	98.6	98.9	(a)	(a)	98.8	98.0	98.8	(a)	(a)	(a)	98.0
I-52	Daza	98.6	98.4	98.6	98.4	98.4	(a)	(a)	(a)	97.6	(a)	98.2	98.0	96.8	98.0
I-53	Baltazar[d]	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
I-54	Acosta	(a)	98.4	98.4	(a)	(a)	98.2	(a)	97.8	97.8	98.9	98.4	98.2	97.8	98.0

No.	Name.	June 17, 1925. Eighth.		June 18, 1925. Ninth.		June 19, 1925. Tenth.		June 20, 1925. Eleventh.		June 21, 1925. Twelfth.		June 22, 1925. Thirteenth.		June 23, 1925. Fourteenth.		June 24, 1925. Fifteenth.	
		a. m.	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
I-23	Torres	97.8	98.8	98.0	98.6	97.8	98.0	96.8	97.6	98.2	98.6	98.0	98.6	98.6	98.0	98.2	98.4
I-24	Rojas	97.8	98.6	(a)	98.0	(a)	(a)	97.8	97.0	(a)	(a)	97.2	(a)	98.2	(a)	97.8	(a)
I-25	Alegrado	97.8	98.6	97.4	97.8	98.2	98.6	98.0	98.2	97.4	98.2	98.0	98.8	98.2	98.4	98.0	98.0
I-26	Asistin	97.8	99.4	98.8	(a)	(a)	98.2	98.2	98.8	(a)	(a)	98.6	(a)	98.4	99.0	97.8	97.8
I-27	Puri	97.8	(a)	97.6	(a)	(a)	(a)	98.0	98.2	97.4	98.0	98.6	98.6	98.4	(a)	98.0	97.4
I-28	Mendoza	98.4	97.8	96.8	98.4	98.0	97.4	96.8	97.4	97.8	97.2	98.2	98.2	97.2	98.0	97.0	97.8
I-29	Buss	98.2	98.8	97.4	(a)	98.6	98.0	98.8	97.8	99.0	98.8	98.2	98.6	98.6	99.0	97.4	98.6
I-30	Ferrer	98.2	96.8	97.8	97.8	(a)	98.4	98.2	97.8	97.4	98.2	96.8	97.0	97.2	(a)	97.8	97.8
I-31	Villamayor	98.2	98.0	98.6	98.0	98.2	98.0	97.2	99.0	98.0	98.0	97.8	98.2	97.2	98.0	99.0	98.6
I-32	Laspiñas	98.4	99.4	98.5	98.6	(a)	97.4	98.0	98.2	(a)	(a)	97.8	97.8	98.0	98.6	98.0	98.4
I-33	Paule	97.8	98.6	100.2	98.6	98.2	98.0	98.2	97.8	100.6	(a)	97.8	98.0	99.0	98.4	98.6	98.2
I-34	Bacani	98.6	(a)	(a)	98.4	(a)	(a)	98.0	97.8	96.6	98.6	98.0	98.0	98.0	97.6	97.0	96.8
I-35	Canlas	98.6	98.8	98.4	98.0	98.2	97.6	96.8	98.0	98.4	98.2	96.2	98.6	(a)	98.4	97.8	97.6
I-36	Cerrano	98.0	98.6	97.4	98.4	98.2	97.4	98.4	(a)	98.0	97.8	98.2	98.4	98.2	98.4	98.0	97.8
I-37	Macasait[b]	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
I-38	De la Rosa	98.2	99.4	98.6	98.6	98.4	98.6	97.8	98.2	97.0	98.4	97.8	98.8	98.0	98.0	98.6	96.8
I-39	David	98.2	(a)	98.6	98.2	(a)	98.4	98.6	99.2	97.4	98.2	96.8	98.2	98.0	98.8	98.6	98.2
I-40	De Castro	97.8	97.6	97.6	(a)	(a)	97.0	98.0	99.0	96.8	(a)	97.2	99.2	98.2	(a)	98.0	98.2

[a] Did not report to have temperature taken.

[b] Discharged from service.

[c] Temperature these two days due to typhoid vaccine.

[d] On detached service, unable to report.

TABLE 46.—*Temperatures of Filipino volunteers four months subsequent to injection of dengue virus*—Continued.

No.	Name.	June 17, 1925. Eighth.		June 18, 1925. Ninth.		June 19, 1925. Tenth.		June 20, 1925. Eleventh.		June 21, 1925. Twelfth.		June 22, 1925. Thirteenth.		June 23, 1925. Fourteenth.		June 24, 1925. Fifteenth.	
		a. m.	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*	*a. m.*	*p. m.*
I-41	Soriano	98.6	98.0	98.2	98.2	98.6	98.2	98.6	98.4	98.2	(a)	98.2	98.6	98.0	98.4	98.0	97.8
I-42	Torio	98.2	98.7	98.0	99.0	98.0	98.0	98.0	98.4	98.0	98.2	97.2	98.4	96.8	(a)	98.2	98.4
I-43	Abad	96.8	99.5	97.8	97.8	98.2	97.4	98.0	98.4	97.6	97.6	98.6	98.2	97.2	98.6	98.0	98.0
I-44	Silvano	98.2	98.6	96.4	(a)	(a)	97.0	97.2	(a)	97.8	97.2	97.8	(a)	98.2	(a)	98.6	98.0
I-45	Nicolas	[c] 100.2	99.2	99.2	98.6	98.4	98.4	98.2	98.0	97.8	98.0	98.2	98.4	98.6	98.4	98.2	98.0
I-46	Lepa'am	98.2	(a)	98.4	98.0	98.6	98.4	97.8	98.8	98.6	97.8	96.8	98.6	98.6	(a)	98.0	(a)
I-47	Mallillin	(a)	98.0	(a)	(a)	98.0	98.4	98.2	98.4	98.2	98.6	97.8	98.5	98.6	98.4	97.0	98.4
I-48	Nicdao	(a)	(a)	97.8	(a)	(a)	98.2	97.2	(a)	(a)	(a)	96.2	(a)	(a)	(a)	97.0	(a)
I-49	Ordunia	(a)	99.1	98.4	98.2	98.2	(a)	97.6	98.4	98.7	98.6	96.8	98.8	98.2	98.6	98.8	98.2
I-50	Trillanes	98.2	98.4	98.0	98.6	(a)	(a)	98.6	(a)	(a)	(a)	98.2	(a)	98.0	(a)	98.0	(a)
I-51	Cawaring	(a)	98.6	98.6	98.2	(a)	98.0	97.8	(a)	98.4	(a)	96.8	(a)	98.8	(a)	(a)	(a)
I-52	Daza	(a)	98.6	97.6	(a)	(a)	(a)	97.8	(a)	98.2	(a)	97.2	98.2	98.6	(a)	97.8	(a)
I-53	Baltazar [d]																
I-54	Acosta	(a)	(a)	98.0	98.2	(a)	98.6	96.8	(a)	(a)	(a)	97.2	(a)	97.6	(a)	98.0	(a)

[a] Did not report to have temperature taken.

[b] Discharged from service.

[c] Temperature these two days due to typhoid vaccine.

[d] On detached service, unable to report.

transitionals, 12 per cent. There is, therefore, no definite evidence that this soldier had dengue; but, considering the fact that he had received a subcutaneous injection of dengue virus and that he had fever on the fourth day thereafter with no other discoverable cause for it, the supposition is at least permissible that this mild pyrexia, which would have been unnoticed if his temperature had not been taken, is a typical example of the abortive attacks of dengue which natives of dengue endemic areas suffer with sufficient regularity to keep up a high degree of immunity in them.

We venture to believe the cases of four other Filipinos in these groups contain points of more than suggestive interest in a study of immunity to dengue. De Castro's temperature went to 99.8° on the eighth day after injection; Soriano had fever on the eighth and ninth days subsequent to the injection; Torio on the sixth and seventh; and Silvano on the sixth and eighth. All these men were questioned very carefully and all stated that they would not have known they had any fever if their temperatures had not been taken. Soriano had some headache on the days of his fever, but he had no pains in his eyes, back, or limbs, and no rash at any time.

Considering the chronology of the brief attacks of fever these men had (that is, the relation of the fever to the injection), it seems permissible to believe that they exhibited a specific reaction of immunity to the dengue virus. This point seems susceptible of proof. If at some time in the near future we can infect a few Filipinos and identify the presence of virus in their blood at the time of their slight febrile attack the evidence will be complete.

The single definite conclusion that can be drawn from the series of injections of virus made into the native Filipino soldiers is that they were all immune to dengue. None of the thirty-two had any symptom clearly indicative of dengue. The records of our work amply prove that under the same conditions white troops recently arrived from the United States would have given a high attack rate. Furthermore, a certain proportion of white troops serving their regular tour here would have had attacks following such injections even if they had had dengue before. The immunity of the native soldiers was greater, therefore, than that afforded Caucasians by one attack of dengue. If we could admit a congenital, racial immunity as being the responsible factor in making the Filipinos refractory to dengue, this would be easily explained. We do not believe such an admission can be made. A few individuals may be naturally immune, but their nationality gives them no advantage in this regard.

It seems not unlikely that the slight, brief, febrile attacks recorded above were reactions of immunity to the dengue virus. This would check with the theory that natives of dengue endemic areas do not enjoy natural immunity, but that the immunity they possess results from repeated attacks of dengue, which generally remain unnoticed because they are mild in character and brief in duration. *Aëdes* mosquitoes are constantly present, and native people are bitten by them frequently. The perpetuation or conservation of the dengue virus in this endemic area, therefore, presents no problem. It is likewise a simple matter to construct a theoretical dengue history for the average native of a dengue endemic area, as follows: His first attack occurs in infancy when diagnosis is difficult and usually not attempted. Thereafter, as a result of frequent reinfection, he has an abortive attack as soon as his immunity has gone down to a point below that which gives him complete protection.

Thus our deductions and theories are not at variance with the belief that immunity to dengue is not permanent but diminishes gradually, and in order that an individual may remain refractory his immunizing forces must be stimulated repeatedly at intervals of greater or shorter length.

Immunity in other diseases.—Without considering the matter seriously, it might be thought that a review of our knowledge concerning the resistance to infection that follows certain other diseases and of the immunological studies that have been made in connection with them should throw some light on our problem. The diseases that would come into consideration here are those which in their etiology, mode of transmission, or clinical characteristics could be placed in a group with dengue. The one that seems most closely related is yellow fever, and many points of similarity to it have been noted elsewhere in this report; in its etiology, infectious jaundice belongs with yellow fever. Pappataci, or sand-fly fever, has undoubtedly been mistaken many times in the past for dengue. Trench fever causes joint and muscle pains and may resemble dengue in temperature and pulse curve, and convalescence may be protracted. Typhus fever and Rocky Monutain spotted fever are insect-borne septicæmias; the immunological work that has been done or attempted upon them has not taken into consideration their probable etiologic agent, and it may suggest lines of work for dengue; for the last reason, we may include tsutsugamushi.

Yellow fever.—It has been commonly believed that one attack of yellow fever confers immunity for life. It will be remembered that in 1898 there was much talk of sending a regiment of immunes to Cuba; that is, persons who had recovered from an attack of yellow fever, and could therefore go into that endemic area with impunity. Upon such questions the opinions of H. R. Carter (1922) represent the best knowledge we possess at the present time. He says:

> Whether any men are naturally insusceptible to yellow fever is a question on which I have no data. An immunity, however, is acquired by an attack. All infections from which recovery takes place must confer an immunity, temporary or permanent, else there would be no recovery. Whether in the case of yellow fever this immunity is temporary or permanent is a disputed point. The majority of recent writers on this subject regard the immunity from an attack as short lived—even ephemeral—and subsequent attacks common, indeed, the rule, in subsequent exposures a few years later. With this view the writer does not agree. He holds that the immunity from an attack of yellow fever is a permanent one, as permanent as that from smallpox or measles. The question is rather a difficult one to determine. The writer has attempted to do so. (Byam and Archibald, 2:1243.)

The French Commission, which studied yellow fever in Brazil (Marchoux and Simond, 1905), found that without treatment the virus blood from a yellow-fever patient became inactive after forty-eight hours; but, if covered with paraffin oil, eight days were required for it to lose its infectivity. It was killed in five minutes at 55° C. Their work led them to suggest that a method of preventive vaccination might be founded upon the administration of virus heated to 55° C. or after storing it under paraffin oil for eight days. The degree of immunity obtained by the injection of such virus was low, but it could be reënforced, and thus made complete, by a small dose of fresh virus. They furthermore found the serum of convalescents to have preventive power.

Within recent years our information concerning the immunology and specific prevention of yellow fever has been much extended, through the work of Noguchi (1919). Since the isolation and cultivation of *Leptospira icteroides* he has prepared a vaccine which he claims has distinct immunizing value. At the International Conference on Health Problems in Tropical

America, held at Kingston, Jamaica, July 22 to August 1, 1924, Cran (1924) expressed his belief that the vaccine has immunizing value and that the serum prepared by Noguchi is also valuable in treatment, if the patient is injected with it early in the disease. This serum is produced through the injection of horses with cultures of *Leptospira icteroides.* The serum is tested for potency by injection into guinea pigs of mixtures of virulent blood or organ suspension and immune serum. It is of interest, from the standpoint of immunity as well as from that of the specific rôle of his organism, that Noguchi has been able to demonstrate the Pfeiffer reaction, using various strains of *Leptospira icteroides* and the serum of convalescents from yellow fever.

Infectious jaundice.—Infectious jaundice is likewise caused by a *Leptospira,* and in many ways the disease resembles yellow fever. It was subsequent to his thorough study of infectious jaundice that Noguchi isolated *Leptospira icteroides.* Noguchi (1922) states that only one author (Oguro) has reported relapses in infectious jaundice; they occurred in 5 per cent of Oguro's cases. In man, as well as in experimental animals, one attack of the disease is followed by lasting immunity. There seems to be no record of a second attack of infectious jaundice in man. The blood serum of man or of animals recovered from the infection exhibits a high degree of specific potency against the organism, and the organism is easily destroyed by the immune serum. The organism is agglutinized by the immune serum and undergoes lysis, both in vitro and in vivo. The Pfeiffer phenomenon is easily demonstrated.

Inada, Ido, and others, in Japan (1916), and Martin and Pettit, in France (1916, 1917, 1919), as also Noguchi himself, have produced specific immune serum high in potency through the injection of horses and sheep. There has so far been no opportunity to use this serum for treatment in man.

Pappataci.—Until the demonstration by Doerr, Franz, and Taussig (1909) that pappataci is transmitted by sand flies, it had undoubtedly been confused with dengue very frequently. Some of the early writers, commonly credited with describing dengue, were almost certainly describing this disease.

Rogers (1909) states that relapses have been infrequently reported, but that immunity ultimately results and is commonly believed to be permanent. Natural immunity does not seem to exist. Doerr and Russ (1909) mixed blood serum from a person who had had pappataci two years before with the virus blood of a patient in the first twenty-four hours of the disease and injected the mixture into a person whom they believed to be susceptible; infection did not result. They repeated the experiment, using as the antiserum the blood of a person who had had pappataci only seven days before. In this case protection was not afforded by the convalescent serum; the volunteer came down with pappataci. In other words, in the brief interval of seven days it seemed that preventive substances were not yet present; but they were present two years after the attack.

Possibly we must take this experiment of Doerr and Russ at its face value; but two years is a long time for immune bodies to remain in the circulating blood and we should require a greater number of cases before accepting the preventive value of the serum as established.

Trench fever.—An important characteristic of trench fever clinically is its tendency to relapses. The American Red Cross Trench Fever Committee (1918) noted many relapses, even after prolonged periods. In one case, no evidence of active disease had been manifested for thirty-nine days; in another, symptoms reappeared after a well interval lasting forty-nine days; another had a period of normal temperature for thirty days.

Byam (Byam and Archibald, 3:2129) says:

> It is obvious, then that THE SEQUELAE OF TRENCH FEVER ARE BUT A CONTINUANCE OF THE DISEASE AND EVIDENCE OF A PERSISTENCE OF THE INFECTION—a finding that must have a far wider bearing on medicine generally, when other neurasthenic conditions are considered, than in the field of trench fever alone.

The protracted convalescence sometimes seen in dengue may, indeed, be due to the continued presence of the virus in the body of the patient. It would be interesting to know whether or not such persons may be infective carriers of the virus. The virus of trench fever is present in the circulating blood, as determined by experiment, during a long period. Is this possibly also true in the cases of dengue with greatly protracted convalescence?

When the trench fever patient finally recovers from the disease a certain degree of immunity, of course, results. One volunteer subject inoculated with 20 milligrams of known infective louse excreta one hundred eighty-two days after his attack had an entirely negative result.

No immunity resulted from injections of killed virus, but Byam (1923) thinks the use of subinfective doses of living virus holds out brilliant prospects.

Typhus fever.—It is doubtful whether or not any persons are naturally immune to typhus fever. In the London and Glasgow Fever Hospitals, according to Arkwright and Wilcox (Byam and Archibald, 3:2085), all nurses not protected by previous attacks contracted typhus within two or three months after commencing work during typhus epidemics. Subsequent attacks are very rare, but they undoubtedly do occur. Several investigators have shown that convalescents do not suffer any ill effects if infected lice are fed upon them. Many attempts have been made to demonstrate the therapeutic value of the blood serum of convalescents, but the results have not been strikingly successful. The same may be said of the attempts that have been made to use as a vaccine the blood from patients taken at the height of the fever. The results have not been very encouraging. Valuable research has been carried out along these lines by Charles Nicolle (1920.) Lebailly and Poirson (1919) tried prophylactic inoculations, using the serum of typhus patients or of infected guinea pigs after the addition of carbolic acid; they likewise immunized horses with typhus virus. Their reports are encouraging, but the numbers of persons injected were not large.

The agglutination of *Proteus* X 19 by the blood of those suffering with typhus fever has positive diagnostic value. There seems to be no reason why this bacillus should be agglutinated regularly by typhus-fever serum and not by the serum of any other infection; it seems merely to be true. The laboratory could render valuable assistance to the clinician if a bacterium could be found which would react similarly with the blood serum in dengue.

Rocky Mountain spotted fever.—There is no instance on record of a second attack of Rocky Mountain spotted fever ever having occurred in man. The immunological studies of which this disease has been the subject have been productive of important results. Ricketts and Gomez (1908) found that guinea pigs could be protected from Rocky Mountain spotted fever through the injection of the serum of a recovered guinea pig, even though the serum were injected two or three days subsequent to the injection of the infected material. These investigators injected horses with large quantities of virus blood from guinea pigs and monkeys; the resulting serum seemed to possess some neutralizing power as compared with that of horse serum, but its potency evidently was not high. Noguchi (1919) was unable to make a serum of practical potency through the injection of horses; he was able to get better, but not satisfactory, results by permitting infected ticks to bite horses.

Noguchi's best results were obtained through the use of rabbits. These animals are susceptible to the infection, but usually they are not killed by it. After recovery they are completely resistant and the immune bodies in their blood can be greatly increased by giving them, about two weeks after recovery, a reënforcing injection of 4 cubic centimeters of guinea pig virus blood. The immune serum is drawn from the rabbits nine days later. It is tested for potency upon guinea pigs. The theoretical dose for man would be about 16 cubic centimeters. So far there has been no opportunity to use it practically, however. It should be pointed out that the method used here is merely an adaptation of that now followed as a routine measure in the preparation of antirinderpest and antihog cholera serums.

Tsutsugamushi.—The immunity acquired through recovery from tsutsugamushi (Byam and Archibald, 3:2149) is not complete. Second and even third attacks in the same individual have been reported, although subsequent attacks are usually less severe than the original. There is no evidence of natural immunity. The disease can be transmitted to monkeys, which are rendered completely immune for at least two or three years following recovery. Attempts have been made to check the development of the disease, or even to diminish its severity through the injection of serum from immune monkeys; but they have not been successful, and the demonstration of immunity principles in the serum of monkeys has not been established, either during the course of the disease or subsequent to it.

DISCUSSION

This brief review brings out clearly three facts which interfere seriously with any hope we may have entertained that a prophylactic vaccine or curative serum of practical value for dengue is likely to result from methods that have been more or less successful in other diseases. These are—

1. The immunity that follows dengue is not uniformly of a solid and lasting type.
2. No laboratory animal is known to be susceptible to the virus, and nothing is known with regard to the ability of any of the animals to produce antibodies to the dengue virus.
3. The etiological agent of dengue has not been isolated.

Furthermore, the indications are that killed virus is incapable of stimulating the production of active immunity.

Since dengue is not a fatal disease, the need for a vaccine or antiserum might seem to be slight. However, when the days of disability caused by it are considered, and the effect a great epidemic might have on a military campaign is brought into the discussion, it is clear that efforts to find a means for producing active or passive immunity are fully justified.

Until the etiological agent is discovered such work as has been done with the *Leptospira* in yellow fever and infectious jaundice cannot be considered. Without a susceptible animal the Rocky Mountain spotted fever work and the typhus work cannot be duplicated with dengue.

There remain, then, the attempts made with virus blood to vaccinate and those made with convalescent serum to secure a curative agent. Such work the French Commission (Marchoux and Simond, 1905) did with yellow fever, Doerr and Russ (1909) with pappataci, and several workers have attempted it with most of the other diseases the etiological agent of which has not been isolated.

The question now arises, shall we investigate the value of such—almost empirical—methods or shall we await the results of the next season's work upon etiology?

If it could be found, a "*Proteus* X 19" for dengue would be valuable.

At the end of the last season, when only a few infective mosquitoes were left (and most of these were wanted for dissection), a few serological tests were made. One of us (A. P. H.) had just recovered from a moderately severe attack of dengue. The infection was probably the result of the bite of one of our own experimentally infected mosquitoes which had escaped.

Mosquitoes, known to be infective, were ground up in 3 per cent caustic soda. After standing for two hours the mixture was neutralized with hydrochloric acid and the undissolved residue was thrown down in the centrifuge. With the clear supernatant fluid as antigen the serum of A. P. H. was used to investigate the possibility of obtaining by this means a specific precipitin test for dengue. In some tubes the two fluids were brought into contact with one another without mixing (ring test); in others they were mixed. The results were not satisfactory, but they were sufficiently encouraging to warrant repetition when another supply of infected mosquitoes can be obtained. Such an antigen might likewise be used with the Bordet complement-fixation test.

LITERATURE

In reviewing the statements made by those who have come in actual contact with epidemics of dengue, the first inpression we get is one of high susceptibility rate of the average population the first time it is attacked. Our knowledge of mild attacks would lead us to suspect that, when 80 per cent of a population is affected so severely that diagnosis is made, practically no individual escapes completely.

Percentage of population attacked.—Bylon, who reported an outbreak of an epidemic disease in 1870, stated that everybody was attacked. Sandwith (1888) saw dengue in a group of houses with a total number of occupants of one hundred eighty-two persons; of these one hundred thirty-seven, or 75.3 per cent, were attacked; later (1890) he stated that dengue often attacks 80 per cent of the population. Godding (1890), in describing an outbreak of dengue in 1885 on H. M. S. *Dragon,* noted that nearly 80 per cent of the crew of four hundred six men came down with dengue. In 1894 (quoted by Cleland, Bradley, and McDonald, 1916) dengue was prevalent on Thursday Island, and nearly every male and the majority of the females came down with it. LeGendre (1911), describing an epidemic at Hanoi, stated that the epidemic affected the larger part of the population, native and European.

In 1885, in Austin, Texas, alone, it is estimated that sixteen thousand out of the population of twenty-two thousand were attacked. Ashburn and Craig (1907) state that seven of the fourteen volunteers who presented themselves for inoculation passed unhurt through an extensive epidemic at Fort William McKinley, and of these two were absolutely immune, three relatively immune, and one doubtful. Of the same number of Hospital Corps men who had not been exposed to dengue, only one was immune. This would agree with the supposition that, of the 50 per cent who seemed to have passed through the severe epidemic, some had actually acquired immunity through mild and unnoticed attacks of dengue.

Natural immunity.—There is no definite evidence in the literature which would permit the supposition that natives of any region are naturally immune. Sandwith (1888), writing of a time when dengue was not endemic in Egypt, says that in a

Turkish harem where there were twenty people, including slaves, all came down with dengue. Those natives were, therefore, no more immune than others; but, in a mixed population (that is, a population where there are large numbers of persons who have migrated from an area of endemicity), a distinct difference in susceptibility is noted. Skottowe (1890) reported an epidemic of dengue in the Fiji Islands which occurred in 1885. He stated that this was probably the first visit of dengue to the Islands, and that a very high percentage of natives was attacked; Indian and Melanesian immigrants suffered but slightly. In describing a denguelike fever in Dutch Guiana, Bonne (1918) stated that three European nurses and the European sailors in the hospital for treatment came down with dengue within a week or two, whereas among one hundred native nurses and other colored attendants there was not a single recognizable case. He noted, furthermore, that mulatto children were not immune.

Relapses and recurrences.—Rush (1818) makes no statement with regard to immunity in the epidemic described by him; there was no opportunity for him to make observations upon it, since the disease lasted only one season. He does say, however, that "in this fever, relapses were very frequent from exposure to rain, sun, or night air, and from an excess in eating or drinking."

Kennedy (1825) says that two attacks may occur in one month. Twining (1826) observed that "many suffer from relapses nearly equal in severity to the first attack, and third attacks have been seen." Raye (1872) observed attacks two to four weeks after the primary attack. Sandwith (1888) saw at the end of November, 1887, two persons with undoubted dengue, both of whom stated that they had had a similar fever two months before. In the previous attack, however, the joint pains were much worse. In addition to these two cases, he heard of many others who had second attacks during that year. Many who escaped dengue in 1880 had the disease again in 1887. Of twelve Anglo-Indians who were exposed to an epidemic of dengue in India in 1874, and in Cairo in 1884, three suffered in India, but not in Egypt; one escaped in India and got the fever in Egypt, and the remaining eight were unattacked by either epidemic. Skottowe (1890) noted that in the epidemic in the Fiji Islands in 1885, recurrences on fresh exposure were not uncommon.

Godding (1890) states that among those on the *Dragon*, there was only one case with two relapses, but in eighteen cases there were undoubted second attacks, and three had third attacks.

Experimental tests of immunity.—Ashburn and Craig (1907) induced dengue in one patient who said he had had three attacks, the last, two and one-half years previously. They knew of a few other cases which developed naturally after a like period.

Cleland, Bradley, and McDonald (1919) failed to produce dengue by the injection of dengue blood into two volunteers who had had the disease twenty-four and two hundred twenty-nine days previously.

SUMMARY AND CONCLUSIONS

This study of immunity to dengue takes into consideration the observations of others who have recorded their findings during and subsequent to epidemics; it gives the figures and certain deductions that can be drawn from them concerning dengue in military organizations stationed in Manila; it includes the details of actual tests for immunity to dengue in persons whose dengue history was definitely known; and it reports a series of actual tests for immunity in natives of an endemic area. All these factors lead to definite and homogeneous conclusions.

1. An attack of dengue is followed by a period of increased resistance to the infection, but the duration of the immunity is exceedingly variable. Certain individuals suffer relapses; others have greatly protracted periods of convalescence; others have multiple attacks, at intervals sometimes surprisingly short. In general, the statement seems justified that subsequent attacks are less severe than the previous ones. In other words, immunity to dengue is not permanent, but diminishes more or less rapidly to a degree which no longer effectively enables the individual to resist the infection.

2. In an area of endemicity, it is likely that native children suffer their first attack of dengue early in life, and that the degree of immunity exhibited later in life is the result of acquired immunity constantly reënforced by frequent bites of infective mosquitoes. In every epidemic season a certain number of native people have clinical dengue. These may be persons who develop immunity with difficulty or who return to the susceptible state rapidly, or who have been removed from infective mosquitoes for a considerable period.

3. In a region where dengue is not endemic, but which is visited periodically by epidemics, the percentage of the native

population attacked will depend directly upon the period elapsing since previous epidemics.

4. Our second epidemiological study indicates that, under military conditions in Manila, persons coming from the United States are attacked during their first year to the extent of about 40 per cent. Of those who have had one attack, 30 per cent may expect a second; of those who have had two attacks, a certain percentage may expect a third; a few will have four attacks, but our figures are not sufficiently large to make a percentage estimate more than suggestive.

5. Experimental inoculations into a group of men who had had dengue at definitely known dates, resulted in second attacks, after intervals of 53, 55, 86, 98, and 121 days. Persons similarly injected after 32, 40, 46, 48, 50, 53, 60, 67, 68, 69, 74, 90, and 94 days were found to be immune. This experimental work confirms the epidemiological study in showing the individuality of the refractory periods which follow dengue.

6. Injections of dengue virus into twenty-nine Filipino soldiers revealed the fact that they were all immune to such experimental infection.

7. A review of our knowledge of immunity to other diseases shows clearly that we cannot transfer any of that knowledge, by inference, to dengue; but it does suggest a few interesting lines of research.

PREVENTION OF DENGUE

INTRODUCTION

The *Aëdes* (*Stegomyia*) *aegypti* mosquito transmits dengue as well as yellow fever, and the investigations made by us show that there are many striking similarities in the mechanism of transmission of the two diseases. The measures to be adopted for the eradication of both diseases are therefore identical. In so far as control measures are concerned, the high mortality rate in yellow fever induces a population to submit to the application of more-drastic measures, and public opinion will approve more readily the allotment of an adequate budget for mosquito control. The prophylactic measures for the control of yellow fever and, inferentially, of dengue, are so well described in text books on preventive medicine and elsewhere in medical literature that their repetition in detail in this report is not considered necessary, and this discussion will be limited to a brief review of those measures that appear to us to be of greatest practical importance.

BASIC PRINCIPLES UNDERLYING AËDES AEGYPTI CONTROL MEASURES

Before discussing specifically the practical measures that can and should be initiated to prevent the spread of dengue, it will be well to review briefly the principles on which they are founded. Chief among these are the following:

Available experimental and epidemiological evidence indicates that dengue is transmitted from person to person only by the adult female of *Aëdes aegypti,* and the mechanism of its transmission is as follows:

The patient with dengue is infective to the mosquito for a short time previous to the appearance of the first symptoms and during the first three days of the disease; for a period of ten days after the virus has been taken into the body of the insect it probably multiplies or passes through developmental stages and not until the eleventh day is the mosquito capable of infecting persons by its bite; once the mosquito becomes infective, it retains this characteristic and is capable of transmitting dengue to human beings for a long period of time, doubtless until its death; the virus causing dengue apparently does not pass from the infected female mosquito through its eggs to the next succeeding generation.

Dengue can spread in a community only when a combination of three factors is present; namely, unscreened cases of dengue to which *Aëdes aegypti* can gain access; *Aëdes aegypti* in such abundance as will permit a considerable number to become infected through biting persons with dengue; and adequate numbers of susceptible individuals exposed to the bites of the infected *Aëdes.*

Aëdes aegypti is one of the most highly domesticated of all mosquitoes, and the following facts relative to its habits and life history must be appreciated to plan effective measures for its eradication:

It apparently does not hibernate and its natural habitats are tropical and subtropical countries. It can and does make its way to colder localities during the summer months and occasionally increases in numbers in such localities, but the advent of cold weather results in its disappearance.

Its activities become restricted when the temperature falls to 68° F. (20° C.) and, when appreciably lower temperatures prevail, 59° F. (15° C.) or less, it becomes sluggish and seldom can be induced to take blood.

On account of its adaptation to the habitations of human beings it prefers to take blood from man.

It is characteristically a day-biter in the Philippines, and probably throughout the world, taking blood between daylight and dusk. It may occasionally bite at night in lighted rooms, but this is the exception rather than the rule.

The span of life of the female of this species is dependent on the multiplicity of natural enemies present in a given locality, atmospheric temperature, weather conditions, vigor of the insect itself, etc. Its average length of life probably does not exceed six weeks. Patton and Cragg (1913) state that under natural conditions it lives six months. Our experience indicates that this is the exception rather than the rule. We have kept females alive in captivity for more than one hundred days and have transmitted dengue to man through the bites of *Aëdes aegypti* that had been infected seventy-five days previously.

Because of its highly domesticated state, its preferential and, practically speaking, its only breeding places are those available in and around habitations occupied by man. The eggs are deposited singly, usually in batches of from twenty to fifty or more, and oviposition is practically always confined to collections of clear water. The eggs are deposited not only on the surface of the water but also along the sides of the container above the water level and they adhere closely thereto. Not infrequently eggs laid on the surface of water will sink to the bottom, but this does not interfere with subsequent hatching. The eggs are very resistant to an unfavorable environment and will hatch after storage for several months in a dry place. When emptying vessels of water in which there are eggs or larvæ, care must be taken to insure the removal or destruction of those clinging to the sides or bottom of the receptacle before more water is added.

In a favorable environment the eggs hatch in two to three days after deposition. The time interval elapsing between the hatching of the egg and the emergence of the adult insect (free-flying stage) depends altogether upon environmental conditions; it may be as short as eight days and may extend to twenty days or more. In the natural habitat of the species (tropical and subtropical countries), under normal conditions, the time interval usually is from eight to fifteen days.

PREVENTIVE MEASURES APPLICABLE TO DENGUE

Methods of procedure adopted for the prevention of dengue should have for their basis the mechanism of its transmission by mosquitoes and the life habits of *Aëdes aegypti.*

Given sporadic cases of dengue, the rapidity and extent of the spread of the disease in a community are dependent on two factors; namely, the number of nonimmunes exposed and the number of *Aëdes aegypti* present in the locality.

Preventive measures follow two lines of attack; the first presupposes coöperative effort on the part of the individual, and the second is dependent on well-organized mosquito-control campaigns on the part of public-health authorities. The latter is the method of election; but, because dengue is mild in character and has no mortality, it is difficult of effective application because of inadequate appropriations. However, economic losses resulting from widespread epidemics of dengue are sufficiently serious to justify the allotment of adequate funds for the prosecution of such campaigns, and public-health authorities should always stress this point. In practice, and particularly in the military service, preventive measures should include both lines of attack.

Much can be accomplished in the control of epidemics of dengue if the individual (including the householder) will lend his coöperation in the following respects:

Persons with dengue should protect themselves from the bites of *Aëdes aegypti* by remaining in bed and under a mosquito net during the first three days of their illness. If dengue is occurring epidemically in a community an individual should strongly suspect dengue if he feels out of sorts, has a headache, has dull, aching pains in the back and other parts of the body, and there is elevation of body temperature.

During epidemics nonimmune persons should use mosquito nets at night, and especially during afternoon siestas.

Houses in which secondary cases of dengue are arising or have occurred recently should not be visited.

When dengue is occurring in a house, its occupants should make unusual efforts to destroy all the mosquitoes about the premises, by swatting, trapping, sprays, etc.

Householders should see to it that water is not allowed to stand in uncovered receptacles in the house or on the premises for a period longer than seven days (vases or other containers for flowers, cans or basins underneath table legs, fire buckets, uncovered water barrels, tanks and cisterns, drip cans, roof gutters, bottles, tin cans, depressions in stumps and plants, bamboo joints, etc.).

Other places where mosquito larvæ frequently occur are the following: In the Tropics, where houses are located very frequently in gardens, mosquito larvæ are found breeding in the leaf axils of such plants as bananas and various species of the so-called elephant's-ear (*Alocasia* sp.); particularly during the dry season, when it is a common practice for gardeners to water plants daily, the leaf axils of such plants will retain some of the water that is daily sprayed over the plants, and such water will serve as a breeding place for *Aëdes* larvæ, when otherwise no standing water is found about the premises.

Needless to say, the preventive measures outlined above are merely palliative as, unfortunately, active coöperation will be forthcoming from only a limited proportion of even the better-educated and more-intelligent section of the population. At military stations that are so situated geographically as to constitute separate and concrete communities, it is possible to accomplish much in the control of dengue along the lines indicated above; the administration is accustomed to coöperation, contacts between the various elements of the population are close, and information and instruction relative to preventive measures can be disseminated effectively and rapidly to all its elements. The method adopted for the dissemination of information to military personnel on duty in the Philippines is indicated in two circulars of information incorporated in the Appendix.

In civil communities (cities and towns) the general statement is justified that epidemics cannot and will not be controlled unless effective mosquito control (antilarval) projects are undertaken and continued under intelligent supervision. Coöperation on the part of the individual as indicated in the immediately preceding pages should be sought merely as a supplementary measure.

The effectiveness of mosquito-eradication campaigns having in view the control of dengue (and for that matter any disease transmitted by mosquitoes) will depend on the thoroughness and comprehensiveness of organization of the project.

To avoid unnecessary and wasteful expenditure of money, time, and energy it is requisite that a considerable number of factors be given careful study preliminary to the actual formulation of the plan of campaign.

The more important of the points requiring consideration are the following:

As to the previous occurrence of the disease in the community, if dengue has been endemic for a number of years, it may safely be inferred that a considerable proportion of the native population has acquired an immunity of greater or less degree.

We have observed in Manila that the native racial groups show a high degree of immunity doubtless resulting from frequent reinfections, and that dengue is confined very largely to the newly arrived foreigner (American or European) who is nonimmune. This observation also applies to yellow fever, and undoubtedly is true for dengue in all tropical areas in which it occurs endemically. In areas of endemicity the rate of flow of the nonimmune population into the area also should be given consideration. If the rate of immigration is small, extensive outbreaks are not likely to occur, and vice versa. In temperate climates, in which the disease is not and has not been endemic, the entire population should be regarded as nonimmune, and epidemics are likely to be extensive and of an explosive nature. Widespread epidemics of this nature, involving hundreds of thousands of individuals, occurred in many of the southern states of the United States of America during 1921 and 1922.

As a general rule epidemics are confined to towns and cities, and this factor requires consideration.

Detailed maps of the town or city are essential, and in areas of endemicity they should show the residential sections occupied by the major part of the foreign element of the population (nonimmunes). In tropical cities such maps should also indicate the parts of the city in which a permanent type of construction for buildings predominates as distinguished from construction of a temporary character (bamboo and thatched houses). Roof gutters are not used in the construction of bamboo and thatched houses, and the inspection of the exterior and surroundings of such houses is readily accomplished by a relatively smaller number of inspectors than would be required for other sections.

Charts showing the daily rainfall, humidity, and temperature are requisite and, if the information is available from the local weather bureau, they should be plotted for preceding years. If the rainfall is light during certain seasons of the year, it may be found that the amount of water that falls during some seasons is so small and rain occurs at such intervals that complete evaporation will occur in three or four days. During such seasons and under such conditions it will be impossible for *Aëdes aegypti* to breed out in casual rain-water containers (bottles, cans, etc.), and the inspection service should be concentrated on its other natural breeding places. Temperature conditions influence the seasonal distribution of dengue in tropical and subtropical countries. For example, the dengue season in the Philippines extends from about April to November, and dengue occurs only sporadically from December to March. In temperate climates, however, where the season of high temperatures is short and comparatively low temperatures prevail throughout the winter, the plan of a campaign to rid a community of dengue may be quite different from that necessary in hot countries. For example, if an epidemic of dengue begins in the late summer months in a town or city in a temperate climate, the inauguration of an extensive mosquito-control campaign for the remainder of that season would involve the needless expenditure of funds, as it may confidently be predicted that the epidemic will come to an end with the advent of cold weather.

Caution must be exercised in the modification of general plans for mosquito-control work on the basis of rainfall and temperature. Careful studies of local conditions are essential, and health authorities should be sure of their facts before discontinuing routine weekly inspections for collections of rain water during dry seasons and before eliminating from consideration the initiation of mosquito-control campaigns on the basis of the advent of cold weather.

Having made a study of the various factors enumerated in the preceding pages and evaluated the relative importance of each, the authorities responsible for the control of the epidemic are in a position to formulate the lines of attack that give promise of most expeditiously and most effectively bringing it to an end.

In mosquito-eradication campaigns coöperation on the part of the individual, as outlined in preceding pages, should always be enlisted to supplement the antilarval control measures. This can be obtained only by educational publicity. Pamphlets somewhat similar to the ones incorporated in the Appendix, outlining the manner in which the individual can lend effectual aid, should be given wide distribution, supplemented by a series of short, carefully prepared, popular newspaper articles explaining somewhat comprehensively the purposes of the campaign. These articles should be written by newspaper writers rather than by medical men, but should be prepared under proper supervision in order that the most important points may be emphasized.

The area to be covered should be divided into districts and if necessary into smaller subdivisions, and adequate personnel (supervising inspectors, inspectors, laborers, etc.) allotted to each subdivision in accordance with existing requirements. Each area must be completely covered by the inspection service at least once a week. An individual card should be kept on file at the headquarters of each district for every house, and on this card should be recorded the potential and actual breeding places found at the various inspections and the steps taken to eliminate them. Consolidated reports showing the status of potential and actual breeding places in each district should be submitted to the central office at weekly intervals. These reports should be consolidated at the central office in order that information may be continuously available as to the progress of the work as gauged by reduction in the numbers of actual and potential breeding places reported.

House-to-house inspectors, before assignment to the inspection service, should have a comprehensive knowledge of the breeding habits of *Aëdes aegypti* and the steps to be taken to eliminate it; namely, destruction; emptying and oiling of water containers; screening of tanks, cisterns, water barrels, etc.; use of fish as natural enemies; exercise of police power, etc. (See also the Appendix.)

Quite recently it has been found that if the usual and common breeding places of *Aëdes aegypti* are eliminated, they will resort to unusual and bizarre localities for deposition of eggs, thus rendering more difficult their eradication. This characteristic has been successfully met and overcome by deliberately providing attractive breeding containers for them, allowing them to deposit eggs at will, and emptying the containers at seven-day intervals.

SUMMARY

1. Dengue and yellow fever are transmitted by the same species of mosquito (*Aëdes aegypti*), and the mechanism of transmission for both diseases is strikingly similar. Epidemics of both dengue and yellow fever are therefore subject to the same control measures.

2. The control of epidemics of dengue usually is accomplished by a material reduction in the mosquito population of the community.

3. The mechanism of transmission briefly is as follows: The dengue patient infects mosquitoes during the first three days of illness; the infected mosquito is able to transmit the virus eleven days after its infection; infected mosquitoes remain infective throughout life; hereditary transmission of the virus does not occur.

4. Epidemics of dengue can occur only when there are simultaneously present cases of dengue fever, large numbers of *Aëdes aegypti,* and large numbers of nonimmune individuals.

5. *Aëdes aegypti* control campaigns must be based upon a consideration of its life habits; it is one of the most highly domesticated of mosquitoes; it apparently does not hibernate; it disappears when the atmospheric temperature falls appreciably below 59° F. (15° C.) and remains there for any great length of time; it prefers to take blood from man; it is essentially a day-biter, but may take blood at night; its average length of life, under natural conditions, is probably not more than six weeks; it breeds by preference inside human habitations and on the premises thereof; it practically always deposits its eggs in collections of clear water; its eggs, being very resistant to an unfavorable environment, retain their vitality after storage in a dry place for several months; the eggs hatch normally in three days and the adult (free-flying stage) emerges ordinarily from eight to fifteen days later.

6. Dengue-preventive measures are based on two lines of attack; namely, coöperative effort on the part of individuals, both sick and well, and organized mosquito-control campaigns on the part of the public-health authorities.

7. Coöperative effort on the part of individuals should include the following factors: Patients should protect themselves from the bites of *Aëdes aegypti* mosquitoes during the first three days of their illness; mosquito nets should be used at night and during afternoon siestas; nonimmune persons should avoid homes in which secondary cases of dengue occur; householders should destroy adult mosquitoes observed within the house; water should not be permitted to stand in uncovered receptacles in the house or on the premises for a longer period than seven days.

8. Consideration should be given to a number of factors preparatory to the formulation of the plan of campaign. If dengue

has been endemic for a long period, many of the natives will have acquired more or less immunity, and epidemics usually are confined to the newly arrived nonimmune foreigner from areas where dengue is not endemic. If the rate of flow of the nonimmune population is small, extensive outbreaks are not likely to occur, and vice versa. In areas of endemicity maps should be prepared showing the following data: Residential sections of the major part of the foreign nonimmune element of population; the parts of the city in which a permanent type of construction for buildings predominates, as distinguished from construction of a temporary character. Charts showing daily rainfall, humidity, and temperature should be plotted. During certain seasons of the year the rainfall will be so low and may evaporate so rapidly that it would be impossible for the *Aëdes aegypti* mosquitoes to breed out in casual rain-water containers. During such seasons and under such conditions, inspections for breeding places should be concentrated on the other natural breeding places of *A. aegypti*. Temperature conditions influence the seasonal distribution of dengue, and in localities in which the temperature falls below 50° F. (10° C.) and remains there for a considerable period of time, epidemics of dengue will necessarily end with the advent of cold weather.

9. In carrying out antimosquito campaigns the principal line of attack should be eradication of the breeding places (antilarval measures), but this should be supplemented by coöperative efforts on the part of the individual. The active interest and support of a moderate proportion of the population can be secured if adequate publicity of an educational nature is carried out. The area to be covered should be divided into districts and adequate and competently trained personnel assigned to each district as inspectors. Inspections should be made at intervals of not more than seven days, and weekly reports relating to breeding places detected should be submitted and consolidated in order that the effectiveness of the work may be evaluated.

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ILLUSTRATIONS

Plate 1

Fig. 1. *Aëdes* breeding jars, showing type of jar used and opening in metal top covered with netting at right.
2. *Culex* mass-breeding. Photographic developing tray used for breeding larvæ.

Plate 2

Fig. 1. Storage cage (front view) used for egg-laying purposes. Jars contain water for deposition of eggs. Petri dishes contain food (aqueous solution of sugar) and water.
2. Storage cage (rear view) for egg-laying purposes. Note sliding wooden door on side, and opening with net sleeve.

Plate 3

Fig. 1. Small breeding cage used for collecting adults as they emerged.
2. Cage for experimental biting. Note sleeve and method of labeling for identification.

Plate 4. Nets, actual size, used on Cages for storing Mosquitoes

Fig. 1. Net that was found to be satisfactory.
2. Net that was found to be unsatisfactory.

Plate 5

Catching mosquitoes for transfer to reserve cage.

Plate 6

Replenishing water supplies, using rubber-capped pipette.

Plate 7

Fig. 1. Lot of infected mosquitoes feeding on experimental subject. The man developed dengue. Mosquitoes can be seen taking blood on inner aspect of foot.
2. Stocks of infected mosquitoes. Breeding and reserve cages also shown on second table, to the reader's left.

Plate 8

Fig. 1. Volunteers being bitten by infected mosquitoes.
2. Entrance to ward. Screened passageway between double vestibules, each vestibule having two screened doors.

TEXT FIGURES

Fig. 1. Floor plan of experimental ward (Ward 16).
2. Chart indicating passage of strains of the dengue virus from man to mosquito through successive generations.

FIG. 3. Chart showing morbidity and mortality rates, disease only, American and Filipino troops (enlisted) on duty in the Philippine Islands.

4. Chart showing admissions, disease only, American and Filipino troops, Philippine Islands, 1904 to 1924, inclusive. Rate per 1,000.

5. Chart showing morbidity rates, dengue fever, United States military troops on duty in the Philippine Islands, 1902 to 1924, inclusive. Rates per 1,000 per annum for American and Filipino troops (enlisted only).

6. Chart showing admission rates, dengue fever, military forces on duty in the Philippine Islands, 1902 to 1924, inclusive. Rates per 1,000 per annum for American troops (enlisted) and for Filipino troops (enlisted).

7. Chart showing morbidity rates for dengue, venereal diseases, and malaria; American troops in the Philippine Islands, 1904 to 1924. Rates per 1,000 per annum.

8. Chart showing dengue rates at military stations in the Philippine Islands, 1922 to 1924. American troops only. Rates per 1,000 per annum.

9. Chart showing dengue curves at six military stations in the Philippines, 1922 to 1924. American troops only. Rate per 1,000 per annum. Same scale used in plotting rates on all charts.

10. Chart showing dengue cases, admission to sick report, American and Filipino troops serving in the Philippine Islands, 1922 to 1924. Rate per 1,000. Total monthly rainfall.

11. Chart showing meteorological data for the city of Manila. Air temperatures and rainfall monthly, 1922 to 1924.

12. Chart showing daily rainfall, Manila, P. I., 1922.

13. Chart showing daily rainfall, Manila, P. I., 1923.

14. Chart showing daily rainfall, Manila, P. I., 1924.

15. Chart plotted from the data in Table 23, showing frequency of incubation periods by days in experimental series of forty-eight cases of dengue.

16. Chart plotted from the data in Table 24, showing the highest temperature reached in the forty-eight experimental dengue cases.

17. Chart plotted from the data in Table 25, showing duration of temperature in the forty-eight experimental dengue cases.

18. Chart showing the percentage distribution of total leucocytes in seventy-five normal cases and in one hundred forty-three cases of dengue.

19. Chart showing the percentage distribution of the differential ratio $\frac{L}{N}$ in normal and in dengue counts.

20. Chart showing the relation of the differential ratio $\frac{L}{N}$ to the total leucocyte count in normal and in dengue counts.

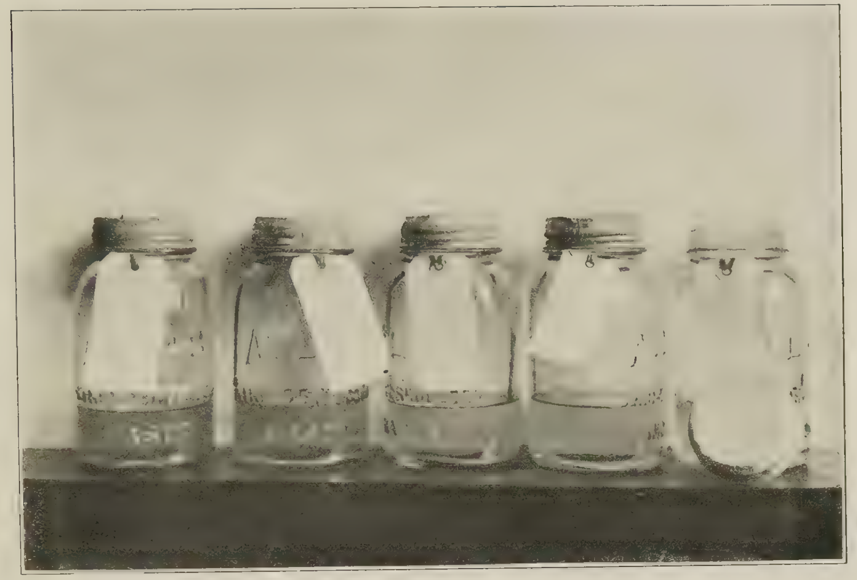

Fig. 1. Aëdes breeding jars, showing type of jar used and opening in metal top covered with netting at right.

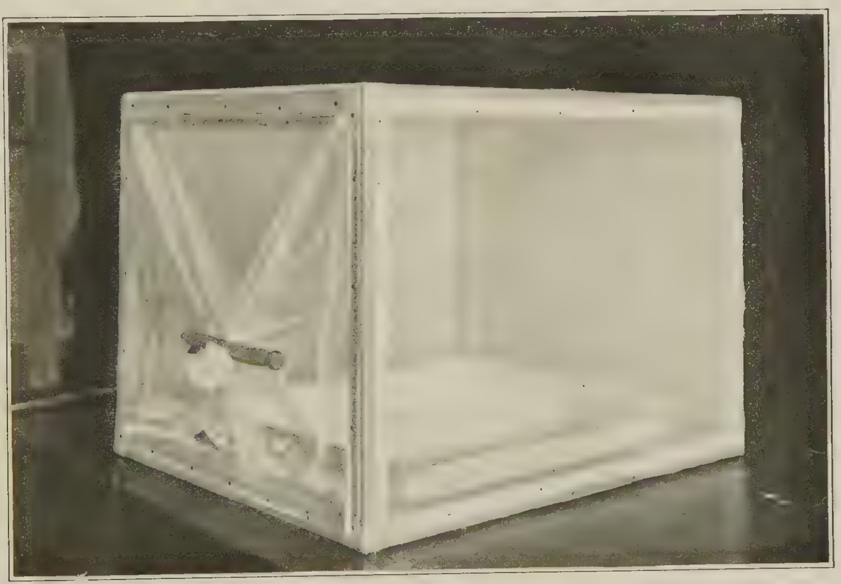

Fig. 2. Culex mass-breeding. Photographic developing tray used for breeding larvæ.

PLATE 1.

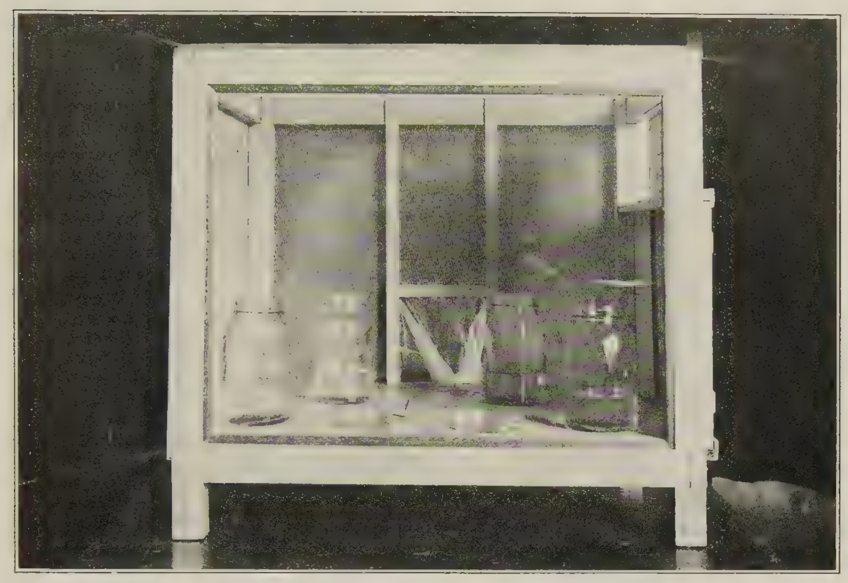

Fig. 1. Storage cage (front view) used for egg-laying purposes. Jars contain water for deposition of eggs. Petri dishes contain food (aqueous solution of sugar) and water.

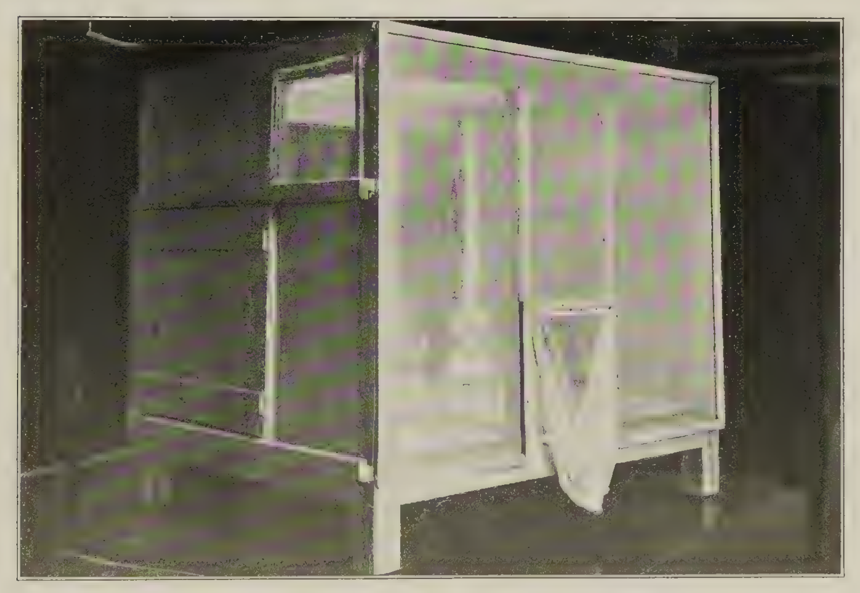

Fig. 2. Storage cage (rear view) for egg-laying purposes. Note sliding wooden door on side, and opening with net sleeve.

PLATE 2.

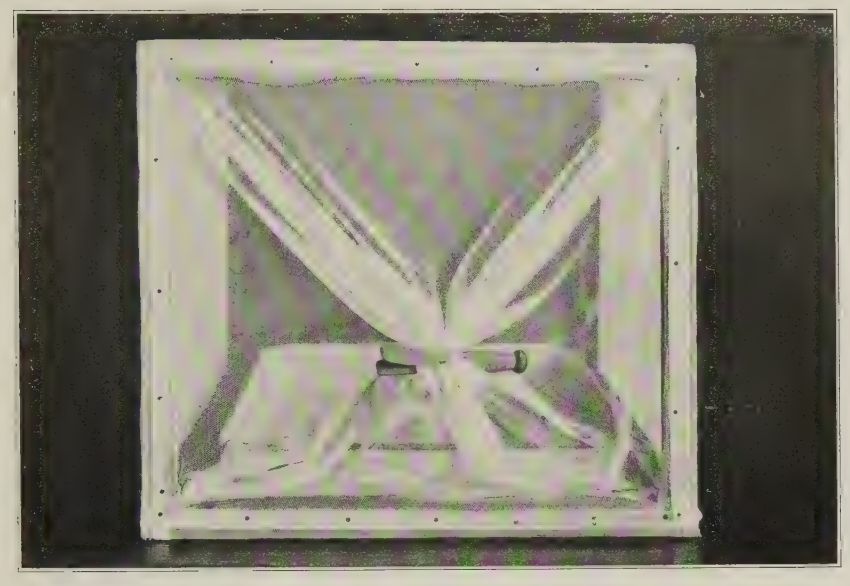

Fig. 1. Small breeding cage used for collecting adults as they emerged.

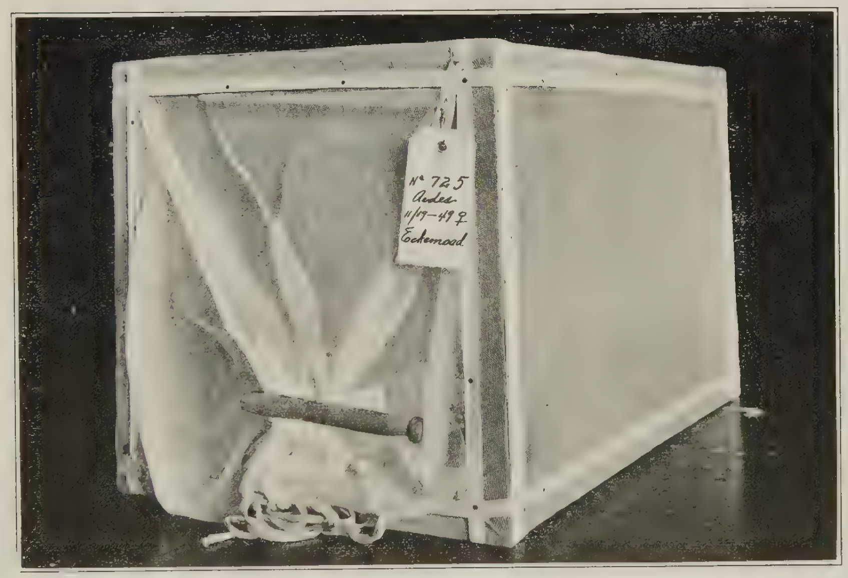

Fig. 2. Cage for experimental biting. Note sleeve and method of labeling for identification.

PLATE 3.

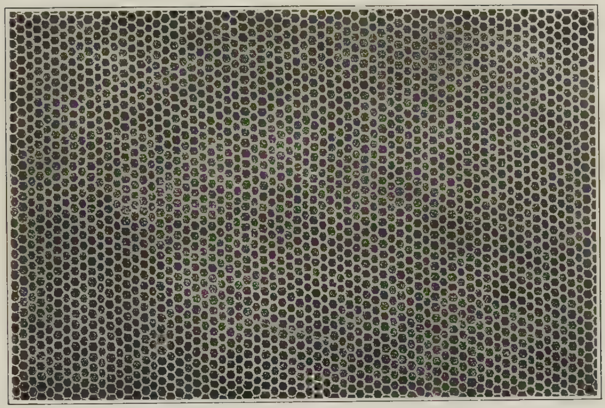

Fig. 1. Net that was found to be satisfactory.

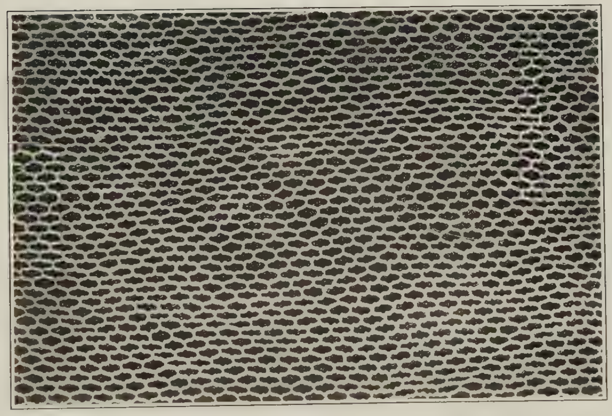

Fig. 2. Net that was found to be unsatisfactory.

PLATE 4. NETS, ACTUAL SIZE, USED ON CAGES FOR STORING MOSQUITOES.

PLATE 5. CATCHING MOSQUITOES FOR TRANSFER TO RESERVE CAGE.

PLATE 6. REPLENISHING WATER SUPPLIES, USING RUBBER-CAPPED PIPETTE.

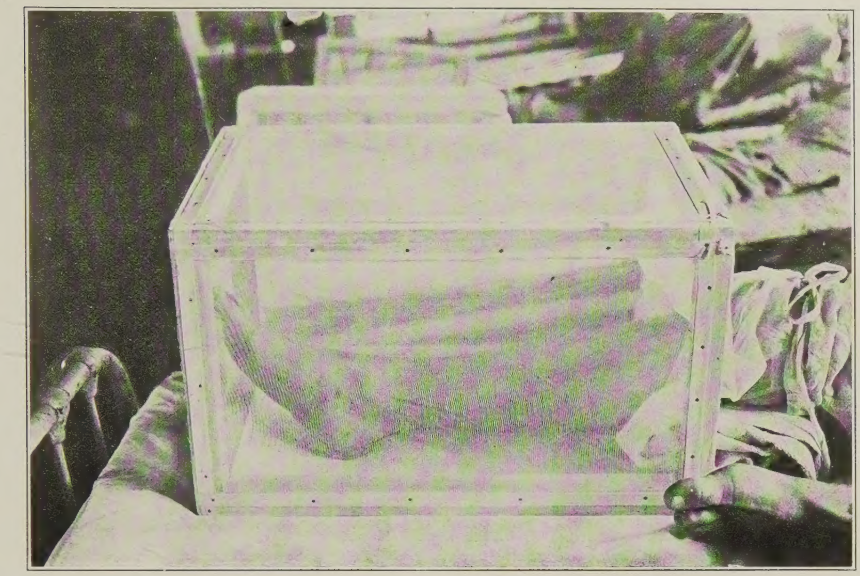

Fig. 1. Lot of infected mosquitoes feeding on experimental subject. This man developed dengue. Mosquitoes can be seen taking blood on inner aspect of foot.

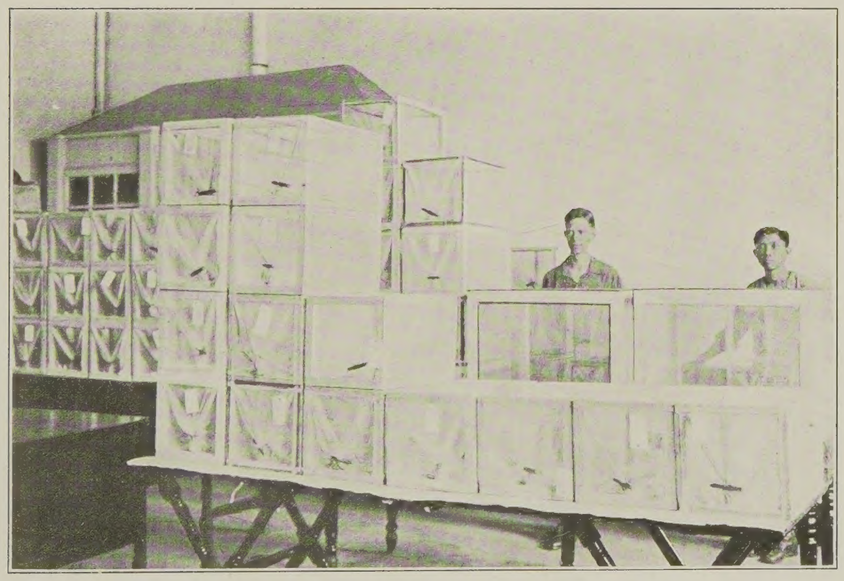

Fig. 2. Stocks of infected mosquitoes. Breeding and reserve cages also shown on second table, the reader's left.

PLATE 7.

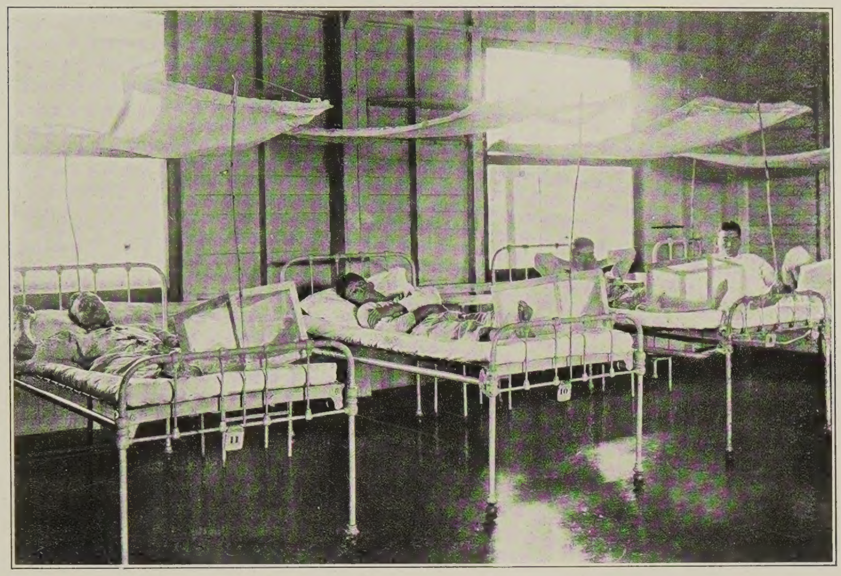

Fig. 1. Volunteers being bitten by "lots" of infected mosquitoes.

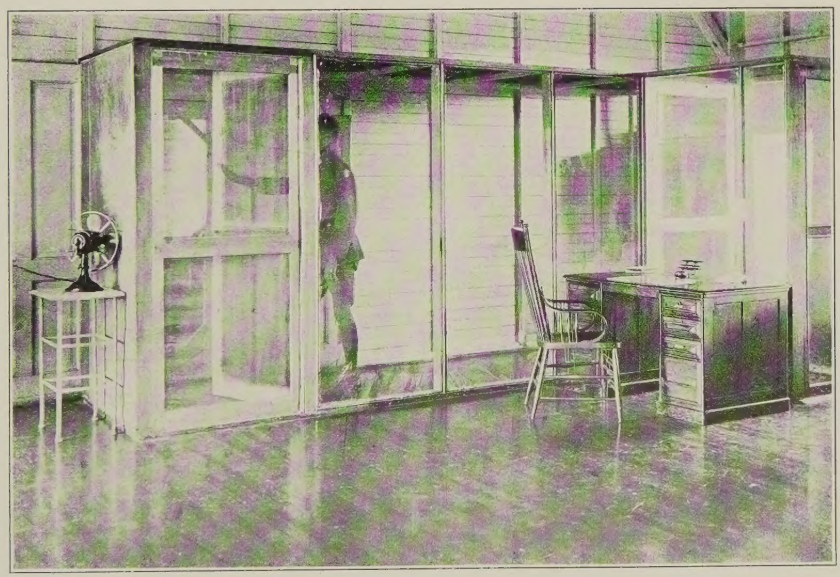

Fig. 2. Entrance to ward. Screened passageway between double vestibules, each vestibule having two screened doors.

PLATE 8